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Contents

Page

Cherry Diet Control for Gout and Arthritis, *Ludwig W. Blau* 309

Quantitative Studies of Cell Types in the Rat Hypophysis Following Prolonged Periods of Unilateral Adrenalectomy, *Rodman Brokaw, Benjamin Briseno-Castrejon and John C. Finerty* 312

Antihistaminics and Motion Sickness Prophylaxis, *Herman I. Chinn, Fred W. Oberst and Syrrel S. Wilks* 320

Legal Aspects of Artificial Insemination, *William D. Decker* 326

Some Electron Microscope Observations on the Structure of Myelin Sheath and Axis Cylinder in Thin Sections, *Donald Duncan and Leland Antes* 329

Preliminary Observations on the Effect of Various Concentrations of Oxygen on the *in vitro* Growth of Spinal Cord from Embryonic Chicks, *E. R. Hudspeth, H. G. Swann, and C. M. Pomerat* 341

Lack of Antidiabetogenic and Antidiabetic Effects of *Tecoma stans* in Alloxan Diabetes, *Joe B. Nash, C. C. Albers, Joyce K. Howard, and Sterling H. Fly, Jr.* 350

Neomycin, a New Intestinal Antiseptic, *Edgar J. Poth, Stanley M. Fromm, Robert I. Wise and Chin Min Hsiang* 353

Proceedings of the Texas Branch of the Society of American Bacteriologists, Spring Meeting, Dallas, April, 1950 361

Observations on the Effect of Certain of the Antimalarial Drugs on Erythrocytes, *R. H. Rigdon and Dorothy Breslin* 371

Effect of Mercurial Diuretics Upon the Respiration of the Rat Heart and Kidney. I. Effect of Mercuhydrin and Its Fractions, *Belle Ruskin, Wiktor W. Nowinski, and Arthur Ruskin* 384

Effect of Mercurial Diuretics Upon the Respiration of the Rat Heart and Kidney Slices. II. The Effect of Thiomerin, *Belle Ruskin, Wiktor W. Nowinski, and Arthur Ruskin* 391

Pathological Diagnoses of Uterus and Ovaries in 6,042 Cases of Pelvic Surgery at Scott and White Clinic, *Frank M. Townsend and John R. Hall* 395

The Effect of Ascitic Fluid and of Blood Serum on the Outgrowth of Epidermal Cells from the Human Skin in Tissue Culture, *O. Ulloa-Gregori, T. G. Blocker, Jr., W. W. Nowinski and C. M. Pomerat* 400

An Inoculating Device for Use in Protozoan Culture, *W. J. Wingo* 410

Integration of Hospital Services and Community Planning Under Public Law 725, *Dean F. Winn* 412

The Emergency Care, Handling, and Transportation of the Injured and Its Importance in the Community, *Carl B. Young, Jr.* 423

Calling Attention To 435

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CHERRY DIET CONTROL FOR GOUT AND ARTHRITIS

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Gout is generally characterized by hyperuricemia, attacks of arthritis, and, in the later stages, by the deposition of urates in the form of subcutaneous tophi on or around the ears, on the joints of the fingers and toes, and on the elbows and knees, as kidney stones or gravel in the kidneys, or after necrosis of the bone in the toes or fingers. Colchicine has been used since the sixth century both for the relief of attacks of gouty arthritis and prophylaxis. Cincophen was found to be effective, but it is highly toxic to some patients. Neocincophen is as effective but without as much indication of toxicity. ACTH has recently aroused great hope in arthritic sufferers. Its expense, however, deters its use.

In presenting the following information regarding observations on a dietary factor in the control of gout and arthritis, apologies are offered for unsatisfactory clinical and laboratory data and control. Nevertheless, the observations made by responsible physicians on the patients concerned suggest the propriety of publishing the information available. In twelve cases of gout, the uric acid of the blood has been decreased to its usual average value, and no attacks of gouty arthritis have occurred on a non-restricted diet in all twelve cases, as a result of eating about one-half pound of fresh or canned cherries per day. Four of these cases report greater freedom of the joints of the fingers and toes. Three case histories are offered in some detail.

Case A. Attacks for over four years. For three years prior to February 11, 1949, an average of one course of colchicine (three or four 1/100 grain tablets daily for from five to seven consecutive days) per month. Two attacks in 1948, in February and in December. Blood uric acid values, during 1948 and prior to February, 1949, determined immediately after each course of colchicine: March 10, 3.0; April 23, 3.4; May 10, 4.5; May 24, 5.2; July 1, 4.5; July 17, 4.6; September 17, 4.6; October 15, 5.8; October 25, 4.4; November 12, 4.6; January 12, 6.15; January 28, 6.4; February 11, 2.3. Attack in February, 1948, lasted over one month. Attack in December lasted two weeks. Started eating cherries

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on February 11, 1949, all canned cherries, sour, black, and Royal Anne. Tired of eating cherries after a few days and has since taken a large glass of cherry juice, obtained by pouring the liquid off cans of water-packed cherries, each day. Has taken no colchicine and no diuretics since February 11, 1949. Blood uric acid values: February 25, 2.18; March 3, 2.2; March 26, 1.78; April 6, 2.1; April 21, 2.2; May 11, 3.22; June 14, 3.25. From February, 1948, to February, 1949, on strict low-purine diet with meat not more often than twice each week. Started eating meat once each day on February 11, 1949; since March 1, 1949, has been eating meat twice each day and has not restricted diet.

Case B. First attack in March, 1935. At least one, and as many as three attacks, each year until July, 1948. Diagnosed as gout in August, 1939. Colchicine taken only during attacks; toxic dose, one 1/120 grain tablet every two hours until ten are taken. Diet restricted. Tophus removed from behind right ear in February, 1948. "Punched-out" area on X-ray photograph of second digit on right foot. First attack, March, 1935, in joint of great toe on right foot. Other attacks in insteps of both feet, and in left wrist. At least two blood uric acid determinations per year since August, 1939. Range, from 4.6 to 9.0. Last severe attack in January, 1948, which lasted three weeks; second attack started within one week and lasted one week. Attack on July 17, 1948, completely relieved within two days by eating fresh Black Bing cherries. Ate non-restricted diet without cherries until August 16, when blood uric acid was 9.0; ate non-restricted diet with one pound of canned sweet and sour cherries each day for three days when blood uric acid was 6.0. This procedure repeated three times, and each time uric acid decreased by eating cherries for three days. Uric acid on February 7, 1949, 1.76. Normal uric acid since on non-restricted diet including cherries three or four days each week.

Case C is presented through the courtesy of Stephen A. Foote, Jr., M.D. This patient, a busy executive, overweight for his age, was first seen on July 24, 1947, when he had his first typical "attack" of gout. There was definite pain and swelling of the first metatarso-phalangeal joint of the right great toe. This was accompanied by fever and he made an excellent recovery with colchicine. The physical examination at that time was not remarkable other than obesity and the local findings. No tophi were noted. July 26, 1947: Complete blood count; R.B.C., 5,950,000; Hgb., 120% or 17.5 grams; W.B.C., 10,200; Polys, 82%; small Lympho, 18%. Urinalysis, 1.022; albumin and sugar negative; microscopic, negative. Kolmer Wassermann and Kahn negative. Fasting blood NPN, 35 mg.%. Fasting blood uric acid, 5 mg.%. Fasting blood cholesterol, 200 mg.%. August 6, 1947: Urinalysis, normal. Fasting blood uric acid, 5.5 mg.%. B.M.R., minus 22. Careful low purine diet; thyroid; colchicine as required: salicylates. August 26, 1947: C.B.C. about same as above. Fasting blood uric acid, 5.5 mg.% (oxalated blood). September 16, 1947: Blood uric acid, 5.5 mg.% (oxalated blood). October 10, 1947: Blood uric acid,

6.7 mg.% (oxalated blood). Blood uric acid, 7 mg.% (serum). Blood cholesterol, 200 mg.%. November 18, 1947: Fasting blood uric acid, 6.8 mg.% (serum). Urinalysis, negative for sugar, albumin, and microscopic. Ten lbs. loss of weight. Clinically better. Colchicine, two tablets, once weekly. March 13, 1948: C.B.C. and urine within normal limits. Fasting blood uric acid, 7.5 mg.% (serum). Fasting blood sugar, 96 mg.%. Fasting blood cholesterol, 175 mg.%. August 10, 1948: Fasting blood uric acid, 6.5 mg.% (serum). Had an attack of gout and took colchicine. Wedding of wife's sister recently. Ate more food. February 25, 1949: C.B.C. and urine within normal limits. Fasting blood uric acid, 5.5 mg.% (serum). Cholesterol, 180 mg.%. Had an attack of gout in early February, 1949. Relieved by colchicine, gr. 1/120. April 27, 1949: Fasting blood uric acid, 5 mg.% (serum). Started eating can of cherries daily on April 8, 1949. May 17, 1949: Fasting blood uric acid, 6 mg.% (serum). October 22, 1949: Stopped eating cherries. October 24, 1949: Noted tenderness and sensitivity in joints of toes. Resumed eating cherries. October 28, 1949: C.B.C. and urine within normal limits. Fasting blood uric acid, 6.3 mg.% (serum). Fasting blood cholesterol, 230 mg.%. Fasting blood sugar, 91 mg.%. The patient is clinically better, although the uric acid is up. He has gained weight as he has been on an average normal diet for the past few months. Patient was last seen January 30, 1950, when he was in good condition.

Prior to July, 1949, in five of six cases of arthritis, without satisfactory histories, in whom the blood uric acid was not determined, the arthritic condition was relieved; no improvement was reported in the sixth case in this series. Since July, a decrease in blood uric acid has been observed in two cases after eating cherries; in both cases, attacks involved the knees and elbows in addition to the toes, insteps, fingers, and wrists, and in both cases the attacks were not relieved completely in the knees despite the decrease to normal of the blood uric acid.

As indicated, the juice of the cherry appears to be as effective as the pulp. While most of the results have been obtained from either the juice or the whole fruit of dark-colored cherries, the sweet yellow and red sour cherries appear to be equally effective. No efforts have been made to identify the active principle involved because no laboratory facilities are available. Keracyanin, the coloring pigment, is suspected, but no source of synthetic keracyanin has been located.

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QUANTITATIVE STUDIES OF CELL TYPES IN THE RAT HYPOPHYSIS FOLLOWING PROLONGED PERIODS OF UNILATERAL ADRENALECTOMY*

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Much work has been done in animals on cytological studies of the pituitary gland after physiological alterations; either normal, such as estrus or pregnancy; or experimental, such as removal of other endocrine organs. The purpose of the present work is to extend a study of the effects of unilateral adrenalectomy on the histological structure of the pituitary and adrenal glands.

In spite of remarkable agreement among present investigators concerning relationship between pituitary gland and adrenal cortex there is lack of agreement regarding the specific cell type in the anterior hypophysis which is responsible for the secretion of the adrenocorticotrophic hormone (ACTH). Until recently most of the studies of this problem had been limited to observations on the histology of the pituitary gland following death from Addison's disease (Kraus, 1927), or in the terminal stages of total adrenalectomy in experimental animals. The most common findings under these conditions have been diminution and degeneration of both acidophiles and basophiles. Crooke and Russell (1935) state that the constant features in pathological adrenal insufficiency are the extreme decline in the number of basophilic cells, a variable reduction in the number of acidophiles, the presence of a series of abnormal basophilic cells and of a considerable number of large chromophobles. Severinghaus (1936) described one case of a woman with Addison's disease in which there was a marked increase in basophiles, but stated that in general Addison's disease seems to be associated with a depletion of basophiles. The possibility that such a disease might be primary in the hypophysis or primary in the adrenals with

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opposite appearances in the cytology of the hypophysis must be considered here. Reese, Koneff and Akimoto (1939), in studying the anterior pituitary glands of male rats showing acute symptons after double adrenal ablation, found diminution in number and size of acidophiles correlated with progressive loss of granular material and regressive changes in the Golgi apparatus. Since all these observations were on terminal stages of bilateral adrenalectomy in experimental animals, or on autopsied cases of Addison's disease, the question may be raised as to whether the changes were a result of modification in pituitary-adrenocortical relationship, or of abnormal metabolism of the body as a whole.

Heinbecker and Rolf (1944) in studies on the dog, found that following hypophysectomy, progressive atrophy of the adrenal cortex occurred. Studies were also made on so-called "puncture" dogs in which the infundibular stem was severed through the oral approach, and then with a special instrument a puncture wound about 2.5 mm. in width was made in the midline in the posterior hypothalamus deep enough to sever the fibers running caudally from the paired paraventricular nucleus. The median eminence was also scooped out. Results showed that under such conditions the acidophiles are maintained, the basophiles decrease in number, the adrenal cortex remains normal while the gonads and thyroid glands atrophy. This result suggests that the acidophiles may be the source of the ACTH, and supports the concept of basophilic source for FSH and thyrotrophin.

On the basis of clinical findings in cases of various types of endocrine disorders, Albright and Elrick (1948) attribute all of the hypophyseal hormones, except FSH, to the acidophiles. Daughaday, Perry and McBryde (1950) correlate the acidophiles with ACTH secretion because of their findings of increased adrenal function in acromegaly, a condition which is usually a result of acidophilic tumors of the hypophysis.

In opposition to these conclusions, D'Angelo, Gordon and Charipper (1948) found that absolute adrenal hypertrophy in guinea pigs following food deprivation was accompanied by progressive loss of acidophiles and an increase in the number of basophiles and chromophobes. These results were interpreted as an indication that the basophiles are the source of ACTH, but the results have not been confirmed in other animals. Schweizer

and Long (1950) studied the histology of transplants of pituitary tissue in hypophysectomized guinea pigs and found in the two cases which maintained the adrenal glands best there was a predominance of acidophile cells in the implants. In some other animals there was a predominance of chromophobes, and they conclude that the question of which cell type secretes ACTH is still unsettled.

The response to unilateral adrenalectomy is compensatory hypertrophy of the remaining gland. Since this does not occur in hypophysectomized animals it appears to be dependent upon an increased secretion of the adrenotrophic hormone, and under these conditions, Finerty and Briseno-Castrejon (1949) have found a concomitant rise in the percentage of acidophiles. When unilaterally adrenalectomized rats were subjected to lowered environmental temperatures as an additional stress these changes in acidophiles were accentuated along with increased hypertrophy of the adrenals. It is believed that reduction in circulating adrenal hormones, either by removal of adrenal tissue or by stress, stimulates an increase in the number of pituitary acidophiles and that these cells secrete ACTH. The present work is an attempt to determine the effects of a more prolonged period of unilateral adrenalectomy.

Procedure

Quantitative histological studies of the pituitary and adrenal glands of 126 male rats of the Sprague-Dawley strain were made. Male rats were used to avoid complications of any cyclic phenomena associated with the estrous cycle. The left adrenal was removed at 31 days of age in one-half of the animals by means of an incision in the dorsum within the angle formed by the last rib and the vertebral column. All animals were kept at laboratory temperature (approx. 26°C.). Autopsies were performed 5, 10, 15, 25, 35, 50 and 75 days following unilateral adrenalectomy. The hypophysis was removed by raising the calvarium, lifting the brain upward and backward to expose the optic tracts and adjacent area. Dura surrounding the gland was carefully removed and the gland picked up on a piece of paper. It was fixed immediately in Zenker-formol solution. Adrenal glands and thymus were removed, freed of fat and

connective tissue, weighed on a torsion balance and fixed in 10% formalin.

The pituitary glands were serially sectioned in a horizontal plane at 4 micra and stained with a modified azocarmine stain as developed by Briseno-Castrejon and Finerty (1949). After this stain, acidophilic granules are purplish red, basophilic granules are light green, mitochondria are orange red, red blood cells are brilliant orange and the chromophobes show little or no colorless to pale green cytoplasm. Nuclear membranes are sharply defined and the Golgi apparatus shows as a negative image in both alpha and beta cells. Quantitative determinations of the percentages of each cell type were made by differentially counting all the cells in every fifth field through three equidistant sections in each gland. In some of the later stages only one section through the middle of the gland was used, since in other experiments it has been observed that no significant difference exists between the two methods.

The adrenals were sectioned on a freezing microtome at 15 micra and stained with Sudan IV and hematoxylin. In this process the tissues are not dehydrated or imbedded in paraffin, but are placed directly on the microtome from the fixative. The sections were placed in 70% alcohol for a few seconds, transferred to Herxheimer's solution (1 gm. Sudan IV, 50 ml. 70% alcohol, 50 ml. acetone) for 2–5 minutes and rinsed in 70% alcohol. They were then washed in distilled water, counterstained with Harris' hematoxylin and washed in tap water. The sections were mounted in glycerine and examined for patterns of fat distribution.

Results and Discussion

The results are summarized in Table 1. It can be seen that a marked compensatory hypertrophy of the right adrenal gland occurred within five days following the operation and was maintained for the duration (75 days) of the experiment. As has been demonstrated previously (Finerty and Briseno-Castrejon, 1949), there was no change in percentage of basophilic cells of the hypophysis, but a consistent increase is apparent in the percentage of acidophiles during the first 25 days. The prolonged period following operation in the present experiment

demonstrates the return to a normal level of acidophiles, approximately 50 days after the onset of relative adrenal deficiency. This phenomenon is shown graphically in Fig. 1.

The response of the pituitary-adrenal axis in these experiments probably may be classified as the Type 2 response suggested by Sayers and Sayers (1949). This type involves a gradual change in internal or external environment, in which a gradual increase in demand for cortical hormones results in a gradually increased secretion of ACTH. Adrenal constituents

TABLE 1

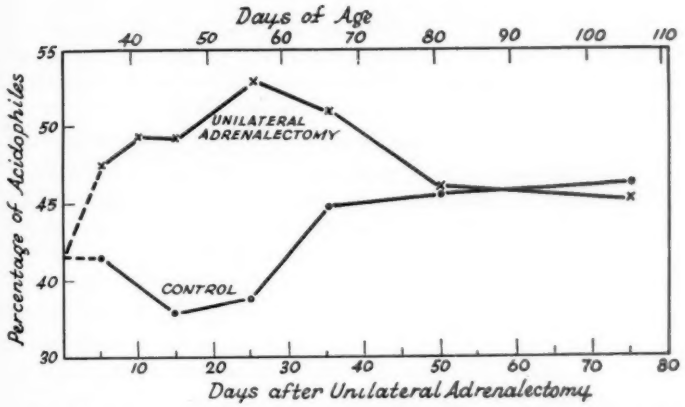
The Effect of Unilateral Adrenalectomy on Compensatory Adrenal Hypertrophy and Percentage of Cell Types in the Pituitary Glands of Immature Male Rats

	Days After Operation	Number of Rats	Right Adrenal Wt. (mg.)	Adrenal Hypertrophy[1] %	Pituitary Cell Type Analysis Acid. %	Baso. %	Chrom. %
Control		7	12.1		41.5	7.3	51.2
	5						
Operated		7	15.6	29	47.4	7.1	45.5
Control		7	14.5		39.0	7.5	53.5
	10						
Operated		7	18.8	30	49.5	7.7	42.8
Control		27	16.3		38.0	7.4	54.6
	15						
Operated		27	19.7	21	49.1	7.3	43.6
Control		7	16.4		38.8	7.4	53.8
	25						
Operated		7	20.8	27	53.0	8.1	39.9
Control		5	20.5		44.7	6.5	48.8
	35						
Operated		5	23.9	17	51.0	6.3	42.7
Control		5	21.9		45.6	6.4	48.0
	50						
Operated		5	27.3	25	45.9	6.4	47.7
Control		5	21.4		46.1	6.4	47.5
	75						
Operated		5	31.3	46	45.2	6.0	48.8

[1]The percentage of hypertrophy is calculated by using the mean weight of the right adrenal glands of the control rats as the basis for comparison.

(sudanophilic substance, cholesterol esters and ascorbic acid) remain unchanged, with a gradual increase in size of the gland. The onset of cortical deficiency in this work is sudden, but it is not an absolute deficiency, is in no case fatal, and may be considered a rather mild, but prolonged stimulation of pituitary

FIGURE 1.



Graphical demonstration of the effects of unilateral adrenalectomy for periods from 5 days to 75 days on the percentage of acidophilic cells in the anterior pituitary gland. On the basis of other work it can be assumed that all rats had approximately the same cytological conditions at the time of operation and that no significant change occurred from the age of 31 to 36, so the dotted lines indicate the probable condition preceding the actual counts begun at 36 days of age (5 days after time of operation).

ACTH secretion. During the first few weeks of this chronic adrenal cortical deficiency there is continual excess ACTH secretion, resulting in compensatory adrenal hypertrophy. It is during this period that the increased percentage of acidophiles is maintained. Statistical treatment of the 15 day group, which includes 27 control and 27 adrenalectomized rats demonstrates that this increase in acidophile percentage is statistically significant. The percentage of acidophiles of the control group is 38.0 ± 0.65[3]; of the adrenalectomized group is 49.1 ± 0.73. The standard error of the difference between the two means is 0.98; and since the difference between the means is eleven times this figure, the difference must be considered highly significant. Additional supporting evidence for the idea that the acidophiles are the source of ACTH is their further increase in percentage and accentuated compensatory hypertrophy when

[3]Standard error.

unilaterally adrenalectomized rats are exposed to cold as an additional stress (Finerty and Briseno-Castrejon, 1949). At about 50 days after operation the pituitary cytological picture regains a normal level, suggesting that the remaining adrenal has reached a state of equilibrium so that it is now a complete functional replacement for the extirpated gland.

There is some indication in the control rats in this series that at about the age of 60 days there is an increase in the normal percentage of acidophiles up to about 45 per cent, and a slight decrease in percentage of basophiles. This may be correlated with the onset of sexual maturity at about this age.

No significant difference from the controls was found in sudanophilic lipid distribution in the adrenals at any period post-adrenalectomy. Although the activity of the hypertrophied gland is certainly increased, there is no apparent depletion of sudanophilic material (at least from 5 days after operation onward). This also fits in well with Sayers' Type 2 reaction in which the gradual changes result in constant stimulation of the adrenal cortex with maintenance of adrenal constituents.

It is concluded from these experiments that 1) the compensatory hypertrophy of unilateral adrenalectomy is a result of heightened secretion of ACTH from the pituitary acidophilic cells; and 2) when the remaining adrenal hypertrophies to become a complete functional replacement for the missing gland (at about 50 days post-operative in this project), the pituitary cytological picture returns to normal.

Summary

The consistent responses following unilateral adrenalectomy of immature male rats are 1) compensatory hypertrophy of the remaining gland and 2) an increase in percentage of pituitary acidophilic cells. Approximately 50 days after the operation, however, the hypophysis returns to a normal cellular percentage, probably due to the attainment of complete functional replacement by the remaining hypertrophied adrenal gland. It is suggested that the increased ACTH activity following reduction in circulating adrenal cortical hormones, which results in the compensatory hypertrophy is mediated by an increase in number and activity of the acidophiles.

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ANTIHISTAMINICS AND MOTION SICKNESS PROPHYLAXIS

HERMAN I. CHINN, FRED W. OBERST AND SYRREL S. WILKS*

The possibility that histamine antagonism might play a significant role in the prevention of motion sickness was first suggested by the findings of Gay and Carliner (1949) who reported the ineffectiveness of Dramamine (beta dimethylaminoethyl benzohydryl ether 8-chlorotheophyllinate) in preventing seasickness. Strickland and Hahn (1949) demonstrated a similar, but less marked, protective action of Dramamine against airsickness. It has recently been shown (Chinn and Oberst, 1949) that the active portion of Dramamine is the diphenhydramine nucleus which is also common to Benadryl (beta dimethylaminoethyl benzohydryl ether hydrochloride). No difference could be detected in the protective action of Dramamine or Benadryl (Chinn and Oberst, 1949). In addition to their antihistaminic properties Benadryl and Dramamine possess other pharmacological properties, of which their anticholinergic action is probably the most significant. It seemed important, therefore, to test other antihistaminics and anticholinergic drugs in order to evaluate the pharmacological basis of motion sickness prevention.

Experimental Methods and Results

The drugs were tested on persons in the airplane by the simulated turbulence technique of Strickland and Hahn (2). Medication was given one hour prior to take-off in a C-47 (DC-3 type of plane). Mild and moderate turbulence, interrupted by short periods of smooth flying, was simulated by the pilot for one hour. The response of the subjects was recorded from direct observation and the side effects obtained by means of post-flight questionnaires.

In addition to testing drugs in the air, motion sickness was also induced on the ground by means of a standardized swinging

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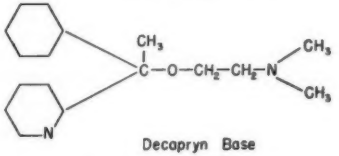
exposure. The drug was again given one hour prior to the start of the swinging. The subject sat erect, with his head supported in vertical position by a head rest. The radius of the swing was 14 feet and the trajectory an arc of 150°. Swinging continued for 20 minutes, unless vomiting occurred earlier.

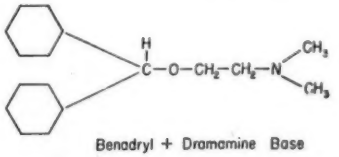
Decapryn (2 alpha (2 dimethyl amino ethoxy) alpha methyl benzyl-pyridine 8-chlorotheophyllinate*) was studied in the plane because of its chemical similarity to Dramamine and Benadryl. As is evident in Figure 1, it differs basically from

Figure 1—Basic Structure of Decapryn & Benadryl



Decapryn Base

2 [α (2 dimethylaminoethoxy) α methylbenzyl] pyridine



Benadryl + Dramamine Base

β dimethylaminoethyl benzohydryl ether

these compounds only in the substitution of a pyridine for a benzene ring and the addition of a methyl group. The comparison of the effectiveness of 30 mg. Decapryn salt versus placebo is shown in Table I. Decapryn exerted a slight protection as evidenced by the lowered incidence and a delay in the onset of vomiting. The decreased incidence of vomiting was not at a statistically significant level, but the delay in the time of onset among those who vomited was significant at the

*Kindly prepared for us and supplied by Doctor Robert Shelton, William S. Merrell Company, Cincinnati, Ohio.

1 per cent level, as determined by a non-parametric significance test.

TABLE 1

Effectiveness of Decapryn in Prevention of Airsickness

Medication	Subjects	*Airsick* No.	*Airsick* %	Time of Onset (Min.)	% Protection
Decapryn 25 mg.	48	23	47.9	28.8*	11.6†
Placebo	48	26	54.2	24.3	

*Statistically significant delay at 1 per cent level.
†Not significantly different from placebo.

Chlor-Trimeton (1-(p chlorophenyl) -1- (2 prydiyl) 3 -N,N-dimethyl propylamine maleate) was also tested against placebo in a separate series of flights. The recommended dose for this antihistamine is lower than for any other available commercially (Loew, 1950). The low incidence of side effects makes it especially attractive for use with flying personnel if airsickness prophylaxis could be demonstrated. One group received 4 mg. in the manner described above, while an equal number of placebos were used as a control group. The findings are summarized in Table II. The protection afforded by Chlor-Trimeton

TABLE 2

Effectiveness of Chlor-trimeton in Prevention of Airsickness

Medication	Subjects	*Airsick* No.	*Airsick* %	Time of Onset (Min.)	% Protection
Chlor-Trimeton 4 mg.	96	47	49.0	24.2	17.5*
Placebo	96	57	59.4	24.8	

*$P = 0.15$ from chi square test of difference from placebo group.

was not significant at the 5 per cent level. However, the data indicate a slight degree of prophylaxis. Essentially the same results were obtained when Chlor-Trimeton was tested on the swing. The incidence of vomiting was slightly lower than in

the placebo group, but this difference cannot be considered significant.

The effectiveness of certain drugs (atropine, hyoscine, diphenhydramine against motion sickness and Parkinsonism suggest a common mechanism of action, possibly by anticholinergic means (Gair and Ducey, 1950). It seemed fruitful, therefore, to test the promising antispasmodic Artane (3 (N-Piperidyl) 1 phenyl - 1 cyclohexyl 1 propanol hydrochloride) against motion sickness. Unpublished data show that Artane was very effective in relieving seasickness (Chinn, Noell and Smith, 1950). However, the incidence of side reactions was high when strongly prophylactic doses (5 mg. three times daily) were given for extended periods. Since exposure to motion in the plane is generally of much shorter duration than aboard a ship, it seemed possible that protection might be attained without a high incidence of undesirable reactions. Five mg. Artane were given, therefore, and compared directly with 0.65 mg. hyoscine-hydrobromide given simultaneously to subjects aboard the same plane. Under these conditions, Artane proved to be somewhat less effective in preventing vomiting than the usual dose of hyoscine-hydrobromide (0.65 mg.). There was no difference in the time of onset of vomiting.

Thephorin (2 methyl 9 phenyl 2, 3, 4, 9 tetra hydro 1 pyridindene tartrate) has also been used in the treatment of Parkinsonism (Berger, 1949). It was tested on the swing and the incidence of vomiting compared with a placebo group. There was an apparent slight protection but not at a significant level.

Discussion

It is evident that the antihistaminic properties of a drug bear little relation to the anti-motion sickness effectiveness of the compound. Three active antihistaminics (Decapryn, Thephorin and Chlor-Trimeton) have shown only slight protection against airsickness or swing sickness. Similarly, unpublished results (Chinn, Noell and Smith, 1950) indicate that Neoantergan, a widely-used antihistaminic, has no protection against seasickness. Thephorin demonstrated only a slight protection among known motion-susceptible subjects. The prophylaxis of some antihistaminics and the failure of others can be roughly pre-

dicted on the basis of the anticholinergic properties of the drug. The anticholinergic power alone, however, cannot be used to differentiate the various drugs quantitatively. Thus, Benadryl and Dramamine have approximately the same protective capacity as hyoscine and Artane, despite significantly lower incidences of parasympatholytic symptoms (dry mouth, blurred vision, etc.) These variations may possibly be related to differences in central and peripheral action.

Summary

The antihistaminics Decapryn and Chlor-Trimeton exert only slight protection against airsickness. Chlor-Trimeton and Thephorin, another antihistaminic, do not protect against swing sickness. Artane, an antispasmodic, protects against airsickness but to a lesser degree than hyoscine. It is concluded that antihistaminic potency is not related to motion sickness prophylaxis.

Table 3

Effectiveness of Artane in Prevention of Airsickness

Medication	Subjects	*Airsick* No.	*Airsick* %*	Time of Onset
Artane 5 mg.	87	42	48.3	22.1
Hyoscine 0.65 mg.	87	33	37.9	23.1

*P = 0.20 from chi square test of difference from Artane group.

Table 4

Effectiveness of Certain Antihistaminics in Preventing Swing Sickness

Medication	Subjects	*Swing Sick* No.	*Swing Sick* %	Protection	P
Thephorin	89	18	20.3	25.4	0.30
Placebo	88	24	27.2		
Chlor-Trimeton	71	14	19.7	18.9	.50
Placebo	70	17	24.3		

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LEGAL ASPECTS OF ARTIFICIAL INSEMINATION

WILLIAM D. DECKER*

It would appear to be wise for physicians to consider whether heterologous artificial insemination should (1) be prohibited by the penal statutes of the various States, or (2) be sanctioned by legislation legitimatizing the children so begotten with the husband's consent. The physician who acts with the consent of the prospective parents should not later be subject to a criminal or civil malpractice suit by either the woman artificially inseminated, her husband, the donor, or the State.

There has been no legislation defining the rights of the respective parties and the judicial expressions have been few and only touched the surface of the perplexing uncertainties of which the physician and the child are now faced. The uncertainties are well illustrated in a New York case[1] wherein the wife filed a suit to determine her former husband's rights to visit the minor child which had been born to them during wedlock. It was assumed by the court that the mother, plaintiff, had been artificially inseminated by donor (A.I.D.) with the defendant's consent. The plaintiff contended that the defendant had no right to visitation since he was not the father.

The court held that the child had been semi-adopted or adopted by the defendant, father, and that he was entitled to the same rights of visitation as a foster parent who had adopted a child, if not the same rights of a natural parent. The court further said that the child was not illegitimate. However, the court expressly refrained from expressing an opinion as to the property rights of the child.

In Canada a court[2], by dictum, expressed the view that a woman voluntarily submitting to artificial insemination (A.I.D.) was guilty of an adulterous act, by saying,

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[1]Strand v. Strand, 78 N.Y.S.(2) 390 (1948).

[2]Oxford v. Oxford, 58 D.L.R. 251.

'"The essence of the offence of adultery consists, not in the moral turpitude of the act of sexual intercourse, but in the voluntary surrender to another person of the reproductive powers or faculties of the guilty person, and any submission of those powers to the service or enjoyment of any person other than the husband of the wife comes within the definition of adultery . . . it is not the moral turpitude that is involved, but the invasion of the reproductive faculty. . . . Assuming the plaintiff's story to be true, what took place was the introduction into her body by unusual means of the seed of a man other than her husband. If it were necessary to do so, I would hold that that in itself was sexual intercourse."'

It does not seem reasonable that our courts could say a woman voluntarily submitting to artificial insemination (A.I.D.) with the consent of her husband was guilty of the penal offense of adultery, since carnal intercourse is a necessary element in order to incur the offense of adultery, the definition of adultery being statutory.

A trial court in Chicago[3] has held that artificial insemination (A.I.D.) is not an adulterous act. The husband had sought a divorce on the grounds of adultery, alleging that a child had been born to them which had been secured through heterologous artificial insemination.

Our English brethren take a view opposite to that expressed in Strand v. Strand (supra), and to that of the Chicago court, according to a report of a commission[4] appointed by His Grace, the Archbishop of Canterbury. This report is in two sections—legal aspects and theological principle. The report states that heterologous artificial insemination is wrong in principle, contrary to Christian standards and adulterous, and recommends that legislation be enacted to make it a criminal offense. Doctor W. R. Matthews, Dean of St. Paul's, a member of the Commission, refused to sign the report and turned in a minority report. The Commission states that the question which the practice raises has not yet come before the English courts.

Various medical editorials in this country suggest that the legal aspects be ignored because of the secrecy involved. It might be that we could ignore the legal aspects if it were only practiced occasionally, but since it is practiced more frequently

[3]The Trial Court (Chicago, Ill.) 1945.

[4]S.P.C.K. 2s. 6d. net.

each year, the rights of the persons involved should be clarified by legislation. It is my opinion that the most urgent legislation needed is as to assure the legitimacy of the child and its inheritable rights from the foster father. I do not think, under the present law, that the mother is guilty of the offense of adultery. I do not think the donor's position is too precarious at this time. I am of the opinion, however, that the physician's immunity from civil or criminal action is uncertain and should be clarified.

The history of much of our legislation is that it is often enacted after grevious injury to the pioneers who proceeded under the dictates of their own consciences only. The various forms of consent which have been written should by all means be used. However, I do not think they are the answer or that they are sufficient. The only satisfactory method of clarifying the present legal uncertainties is by legislation, because inevitably the question of inheritance from the foster father of a child procured by artificial insemination (A.I.D.) will come before our courts. It can only inherit from the foster father if legitimate. The law making bodies of the various states should enact legislation for the guidance of the courts, because of the varying views on the theological, moral and psychological aspects.

SOME ELECTRON MICROSCOPE OBSERVATIONS ON THE STRUCTURE OF MYELIN SHEATH AND AXIS CYLINDER IN THIN SECTIONS

DONALD DUNCAN AND LELAND ANTES*

The recent papers of Fernández-Morán (1950) and of Schmitt and Geren (1950) have added much to direct visual information on the finer organization of myelinated nerve fibers. They have confirmed the pioneer electron microscope study of Richards, Steinbach, and Anderson (1943) on the filamentous appearance of axoplasm and their papers agree in localizing the "neurotubules" of DeRobertis and Schmitt (1948) to the sheath of the nerve fiber rather than within the axis cylinder as originally postulated.

In addition, Fernández-Morán has carried direct visual analysis of both sheath and axon far beyond any previous study. For the most part his findings constitute a remarkable confirmation of information and impressions gathered from the methods of light microscopy, polarization optics and X-ray diffraction studies. In only one respect does his account seem to differ with the more generally accepted concepts; namely, as to the presence or absence of an axolemma, a sheath immediately investing the axis cylinder. Fernández-Morán has interpreted his electron microscope pictures as evidence for such a sheath and this seems contrary to the findings of De Rényi (1929) on micro-dissection of living material and contrary to the usual interpretation of classic histology. In contrast, the results obtained in this study do seem to agree with those who deny the existence of such a membrane. In other respects the present study is in general accord with recently published accounts on the structure of the myelinated fiber as seen with the electron microscope.

MATERIALS AND METHODS

Various peripheral nerves of human, cat and mouse were used. The human material was obtained from the amputated

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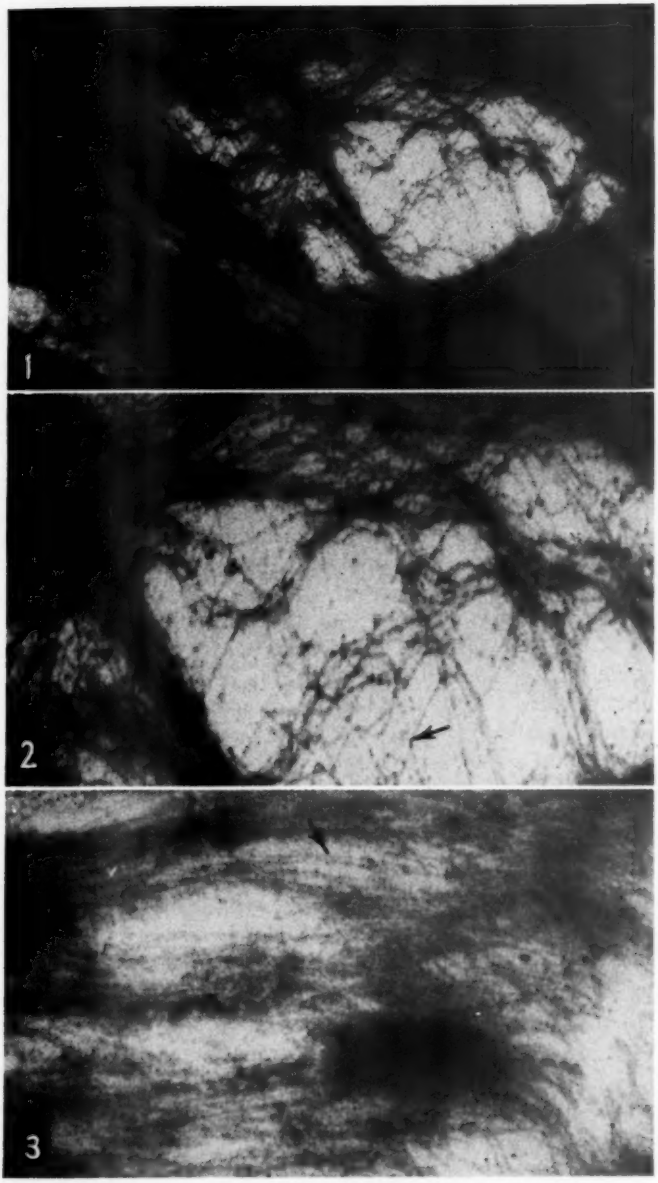
leg of a fifteen year old healthy boy and fixation was started within 30 minutes after removal of the leg. The mouse and cat nerves were removed during terminal etherization of the animals. All nerves were fixed under slight tension by immersion in 1–2% osmium tetroxide solution for 12–24 hours. The fixed nerves were embedded in n-butyl methacrylate according to the method of Newman, Borysko and Swerdlow (1949). Sections were cut with a Spencer rotary microtome containing the reducing wedge of Pease and Baker (1948). The best sections were obtained by applying ice to both knife and block. The sections were cut a few at a time, transferred with a camel's hair brush to an ice chilled glass slide which was moist with condensation of atmospheric water. The sections were then immediately separated and unwrinkled as much as possible with needles under a dissecting microscope. With the microtome set at 0.2 u, serial sections of apparently uniform thickness were obtained frequently. With the machine set at 0.1 u, sections were always irregular in thickness and only an occasional very thin one was obtained. After drying on glass slides at 37°C. the sections were soaked in acetone, again dried and then coated with 1.5% collodion in amyl acetate. Selected areas of the collodion film were stripped from the slide under water and transferred to the conventional specimen screens for electron microscope study. The sections were viewed and photographed in an RCA EMU type electron microscope.

PLATE I

FIG. 1. Longitudinal section of the axis cylinder of a myelinated nerve fiber from the phrenic nerve of a cat. Note prevailing longitudinal course of the axonic filaments except in regions of least density where they appear to be tangled. The tangled appearance is presumably due to forces acting during preparation. X 10,500.

FIG. 2. An enlarged view of a portion of Fig. 1. Variations in density along the course of individual fibrils is apparent. Some of the darker regions are due to intersections of filaments as indicated by an arrow, in other filaments regular changes in density are not associated with intersection of filaments. X 28,000.

FIG. 3. Axis cylinder from the same source as Figs. 1, 2, but in a thinner section. Paired filaments superficially resembling a "neurotubule" are indicated by an arrow. A coarsely beaded filament is seen nearby. X 35,000.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Results

The best general views obtained of the axis cylinder are shown in figures 1 and 2. From these figures the filamentous appearance of the axon is most apparent. The filaments seem to be of uniform size and to some extent beaded. Except for their greater numbers and more nearly parallel and longitudinal course they are very similar to the filaments described by Schmitt and Geren. Much of the apparent beading in this specimen is due to a crossing of two or more filaments registering on the plate as an area of increased density. Other densities along the course of the filaments seem to be independent of superimposition of filaments; however, it is possible that some of these densities are due to the presence of another filament which is in focus only at the point of intersection.

In much thinner sections such as shown in figure 3, many of the filaments appear smooth and uniform at first glance but here and there a distinct and unmistakable beading is present. Closer examination of greatly enlarged areas indicates that all of the filaments show periodic changes in density along their course. The finest filaments that can be seen are approximately 100 A° in diameter and of indefinite length. There is at least a tendency for these filaments to run in pairs and when they do the resemblance to the smallest neurotubules of DeRobertis and Schmitt is striking. While anastomosis and directions other than longitudinal are not excluded by study of the material available,

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Plate II

Fig. 4. Cross section of a single myelinated nerve fiber from the posterior tibial nerve of a 15 year old boy. This figure is characteristic for difference in density between myelin sheath and axis cylinder, retraction of axon from sheath in some places and not in others, irregular areas of vacuolization and condensation, granular rather filamentous appearance of axoplasm, individual and clustered particles that are denser than the general axoplasmic substance. X 20,000.

Fig. 5. Cross section of axis cylinder of a myelinated fiber from the optic nerve of the mouse to illustrate the punctate appearance of myelinated nerves in cross section. X 76,000.

Fig. 6. A portion of the axon from a myelinated phrenic nerve fiber of the cat showing characteristic clumps of dense rounded bodies seen in both longitudinal and cross sections of cat nerves. Longitudinal section. X 35,000.

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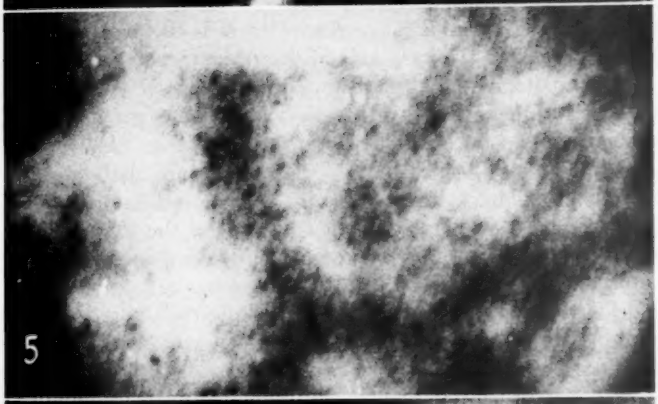
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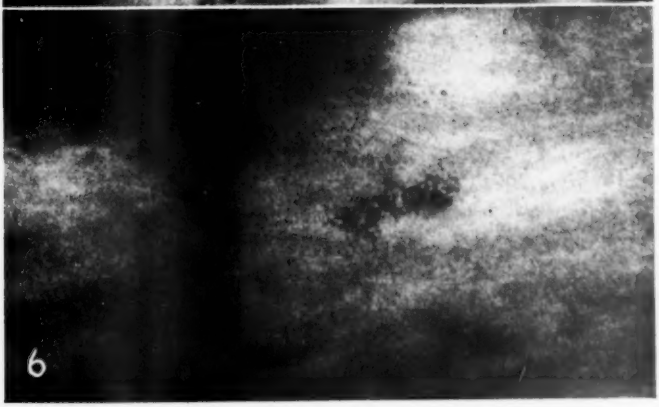
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all preparations strongly suggest individuality of the fibrils and an exclusively longitudinal direction. Deviations from the longitudinal are readily accounted for by fixation artifact. Tangling of the filaments is directly related to clear spaces and these clear spaces suggest separation and accumulation of water during fixation. Such clear and relatively clear spaces present no regular pattern, varying greatly in size and number from fiber to fiber in the same section.

In cross section the filaments appear as individual dots and clusters of dots. Also, they probably compose the amorphous patches in the cross sections that are of the same density as the individual dots and clumps; see figs. 4 and 5. In none of the cross sections is there a suggestion of threads or fibrils running at right angles to the long axis of the fiber. In addition to the main components of the axis cylinder, coarser and denser particles are seen in the cross sections. These particles may be single or in characteristic clumps. Since they have nearly the same density as myelin one cannot be sure that they are not displaced bits from the sheath. On the other hand, they could equally well be normal constitutents of the axis cylinder as the figures suggest. They are not cross sections of fibrillar material since they can be seen in the same punctate fashion in longitudinal sections. Furthermore, the longitudinal sections are devoid of fibrils of a size and density that would be required by the cross sectional appearance. Some of these clusters resemble electron micrographs of viruses, fig. 6, and since they come from presumably normal material they may be indicative of the difficulties that will be encountered in distinguishing normal cell constituents from particles of pathological significance.

Nothing has been seen in the present study that would require an axolemma for its explanation. On the contrary the preparations are highly suggestive of the absence of such a structure. In longitudinal sections the characteristic filaments are seen immediately adjacent to the myelin and no coarser fibrils are located in the zone of transition. In cross section, retraction of the axis cylinder produces the effect of a limiting membrane, see fig. 4, but this is readily explained as a condensation of the retracted material. As mentioned before, the coarser granules seen in cross section are not end views of

longitudinal fibers and their distribution is not limited to the periphery of the axis cylinder.

The myelin sheath is disappointingly dense in sections so far available; consequently, only in a few locations does it appear as anything but a completely opaque ring. Only along knife scratches and in an occasional section cut as a wedge did the electron beam penetrate sufficiently to reveal internal structure see fig. 7. The tendency of the myelin to separate into several relatively course rings as noted by Fernández-Morán is obvious in many fibers. This tendency is, however, much less marked than described by him with the difference probably due to use of frozen sections in one study and methacrylate embedded ones in the other.

All myelin exhibited a fairly coarse lumpiness in both longitudinal and cross sections. On a finer scale concentrically arranged elongated dense masses or flakes are visible in cross sections of mouse, cat and human nerve, figs. 7, 8. For the most part the flecks are too coarse to be considered indicative of molecular spacings. On the other hand they are entirely too regular and conspicuous to be thought of as other than representative of some basic structural pattern. The flecks are largest and most widely spaced in the human material, intermediate in the mouse, and smallest in the cat. In the human and mouse material the flecks follow the contours of the wrinkled and fractured fibers in a manner that clearly rules out diffraction, drift, or aberrations in the lens system. In the cat material the question of artifact is still uncertain. In cross sections of the cat nerves the spacings are not entirely in contour with the fibers, and in longitudinal sections they can be seen running at an angle to the long axis in some sections. In one instance, as shown in figs. 9 and 10 the striated appearance is clearly due to drift of the specimen during photography. In other equally thin places the sheath may appear structureless without apparent reason. Despite these facts, in selected places the fine alternations of dark and light material in the cat may represent the concentric layers of lipoid and protein required by X-ray diffraction studies, particularly since these spacings are also in accord with the flaking and leaflet formation noted by Fernández-Morán. Returning to the conditions seen in the

human and mouse nerves, the flocculent appearance of the lipoid may be an intermediate stage in the disintegration of the myelin, a stage between the approximate living state of evenly spread concentric rings, and the granular or droplet degeneration described and figured by Fernández-Morán. The additional possibility should be entertained that the alternate lipo-protein layers are not uniformly arranged even in the living state but interrupted at regular intervals in both longitudinal and cross sectional directions. The radiating spoke-like appearance of myelin after certain fixatives and the various incisural patterns produced by others seem to require details of ultra-structure in addition to concentric evenly spaced lipoprotein layers. No trace of the radial arrangement of lipoid molecules is seen in these preparations.

Discussion

In evaluating any electron microscope study of tissue, it should be remembered that the material is completely dehydrated and in most current studies the salt content is removed besides. Therefore, what is actually seen is but a skeleton and an altered one at that. Fixatives destroy structure always; some more, some less, and probably always in a selective fashion, see Porter (1950). With these items in mind, and with the rudimentary state of knowledge today concerning their exact effects, any conclusions drawn at present are quite apt to look crude and naive some years hence. On the other hand, appearances

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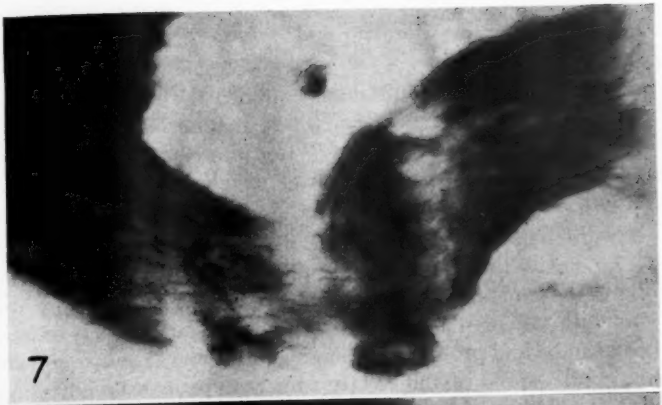
Plate III

Fig. 7. A portion of myelin sheath of a human nerve fiber to show the characteristic appearance of laminated flakes of very dense material. Note that the flakes are irregular as to size and the distances between them. X 17,000.

Fig. 8. Cross section of mouse ventral root fiber showing the same flocculated appearance of the myelin as in Fig. 1 but with greater regularity in size and spacing of particles. X 35,000.

Fig. 9. Longitudinal section of cat myelin sheath showing the lipoid content as linear rows of granules. X 85,000.

Fig. 10. From the same specimen as Fig. 9, but lipoid now appearing as elongated flakes due to drift of the section during exposure of the photographic plate. X 85,000.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seen today should be reproducible under the same conditions in the future however much the interpretation may vary. With these thoughts in mind it may be said that the electron microscope shows a filamentous character to the axis cylinder and a concentrically laminated structure to the myelin sheath.

The filaments seen after osmic fixation and in thin sections are far below the limits of the light microscope and from this follows the conclusion that neurofibrils as seen in fixed preparations with the light microscope are not present as such in life. They can be and probably are aggregates of the axonic filaments seen with the aid of the electron microscope as originally suggested by Richards, Steinbach and Anderson. The present study indicates that the orientation of these filaments is parallel to the long axis of the nerve fiber, probably exclusively so and at least far more so than indicated by the figures of Fernández-Morán and also by those of Schmitt and Geren. The filaments are not consistently uniform in diameter or density although large numbers of them are not conspicuously variable in either respect. Clearly beaded filaments are sufficiently numerous to support the supposition of non-uniformity with the individual filaments composed of regions of alternately dense and light material occurring at 200–500 A° intervals. Anastomosis of filaments is not excluded but appears unlikely. No other constituent is invariably present in all parts of the axon, but unless they prove to be displacements from the sheath rounded particles which are larger and denser than the filaments are present in a random fashion in the peripheral axons of the species examined. Some of these particles occur in characteristic clusters resembling groups of staphylococci on a greatly reduced scale.

Although described by Fernández-Morán, an axolemma is not demonstrated in the present study, and in the authors' opinion the figures of Fernández-Morán are not conclusive on this point. In other words, electron microscope studies to date are interpreted as being in accord with the results of De Rényi on living material and with those histologists who deny the existence of an axolemma on the basis of fixed material studied under the light microscope.

Myelin is undoubtedly a concentrically laminated structure with zones of high lipoid content alternating with those of

another, presumably protein, nature. The ultimate layering may be revealed in certain sections of cat myelin, but a coarser arrangement in which the lipoid does not occur in continuous layers is indicated with certainty. In both the human material and in that from the mouse, the myelin contains elongated flecks of lipoid so arranged as to suggest a discontinuous nature for the lipoid layers even in life. A regular series of ellipsoidal spaces as postulated by Fernández-Morán is partly confirmed in the material here described but is considered questionable as a constant or essential feature of myelin.

Summary and Conclusions

When viewed in thin sections in the electron microscope the main constituent of osmic acid fixed and subsequently dried axis cylinder is a filamentous material composed of non-anastomosing, longitudinally directed, beaded and possible non-beaded filaments. The unit filaments are 100–200 A° across and of unknown length. The beaded appearance of many, if not all, of the filaments is due to alternate regions of electron opaque and electron translucent material. The two densities are of approximately equal length and each occupies from 100–250 A° along the length of a filament. Alterations in density vary as to length in different filaments but seem to be uniform for any one of them. The individual filaments show a tendency to run in pairs and also in bundles that would be visible in the light microscope as neurofibrils.

The axis cylinder also contains dense spherical bodies with a random distribution and occurring singly and in clumps.

No evidence of an axolemma was seen.

Myelin consists of alternate concentric layers of different electron density. Under the conditions employed, the dense material, presumably lipoid, is never seen in continuous layers but always as elongated flecks of varying coarseness.

Note: Since the manuscript of this paper was submitted for publication an article by Rozsa, Morgan and Wyckoff further establishes the filamentous and longitudinally oriented character of the axon and, like the present paper, gives no evidence for an axolemma. See *Science* 112:42–43, 1950.

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PRELIMINARY OBSERVATIONS ON THE EFFECT OF VARIOUS CONCENTRATIONS OF OXYGEN ON THE *IN VITRO* GROWTH OF SPINAL CORD FROM EMBRYONIC CHICKS*

E. R. Hudspeth, H. G. Swann, and C. M. Pomerat

Several studies have been made of the effects of different concentrations of oxygen on the growth of tissues *in vitro*. Burrows (1917) grew heart and skin from embryonic chicks and found very little change in growth in the presence of from about 8% to 100% oxygen. The same author (Burrows, 1921) later found that younger embryonic cells are less susceptible to lowered oxygen tension than older embryonic cells: 4 to 5 day chick heart grew for a short time in a container with pure nitrogen. The minimum amount of oxygen required to permit growth of the 10 day heart was 1.8% while it was 5.4% for the 15 day heart. Mottram (1927) observed slight growth of cells from the Jensen rat sarcoma at oxygen tensions as low as 20 mm. Hg. Assuming that the barometric pressure was 760 mm. Hg. and that the vapor tension was 47 mm., this represents an oxygen concentration of 2.8%. Wright (1928) noted mitoses in growths from explants of 10-day chick hearts in oxygen concentrations as low as 12 mm. Hg. (1.7%); in the case of the Jensen rat sarcoma, mitoses were noted in oxygen concentrations as low as 6 mm. (0.8%); in the case of mouse sarcoma, the minimal oxygen concentration was 3 mm. (0.4%).

Wind (1926) attempted to culture Rous chicken sarcoma under anaerobic conditions. However, accurate determinations revealed the presence of 2×10^{-4} volumes per cent oxygen which permitted good growth in the first passage. Transfer of the tissue to another similar anaerobic culture produced less outgrowth during another 48 hours. No growth was obtained when this procedure was repeated. Using phosphorus for its

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absorption, Wind reduced the concentration of oxygen to 10^{-5} volumes per cent. After 48 hours of growth under these conditions, the cells were proven viable by culture in hanging drop preparations.

These studies clearly suggest that the Jensen rat sarcoma as well as the Rous chicken sarcoma require far less oxygen for growth *in vitro*—for a time at any rate—than fibroblasts from chick hearts. It is, of course, not surprising that different tissues have different requirements of oxygen for growth. But since none of these studies have dealt with growth of nervous tissue and since the oxygen requirement of nervous tissue is known to be very high, the effects of different concentrations of oxygen on the *in vitro* growth of embryonic chick spinal cord were investigated.

Materials and Methods

The technique utilized for studies on nervous tissue was designed to insure that the explanted tissue would reach equilibrium with the gas conditions of the experiment as soon as possible after removal from the animal. Gas mixtures were made in a spirometer and stored in Douglas bags immediately prior to each experiment. The mixtures which were used are listed in Table I. To assure asepsis, the gases were passed through cotton filters. The setup utilized is represented in Figure 1. Gas from the Douglas bag (A) was directed into a glass cup

TABLE I

The Effect of Gases on Embryonic Spinal Cords in Tissue Culture

Gas Mixture	Density of fibers at 48 hours	Number of migrating cells at 48 hours	Amount of liquefaction at 72 hours
96% O_2, 4% CO_2	Abundant	Few	Slight
50% O_2, 4% CO_2, 46% N_2	Many	Many	Moderate amount
14% O_2, 5% CO_2, 81% N_2	Many	Many	Moderate amount
2% O_2, 4% CO_2, 94% N_2	Few to none	Greatest number	Greatest amount
4% CO_2, 96% N_2	None	None	None

(B), which contained Tyrode's solution, by means of a bent tube designed to prevent back siphonage. Using the technique described by Pomerat, Drager and Painter (1946) as a basis, the spinal cord of a 9-day chick embryo (C) was quickly transferred into the Tyrode's solution through which a given gas mixture was bubbling. The cord segments were then removed from the gassed solution, cut into cross sections (D) approximately 1/3 mm. thick, and returned to Tyrode's. After bub-

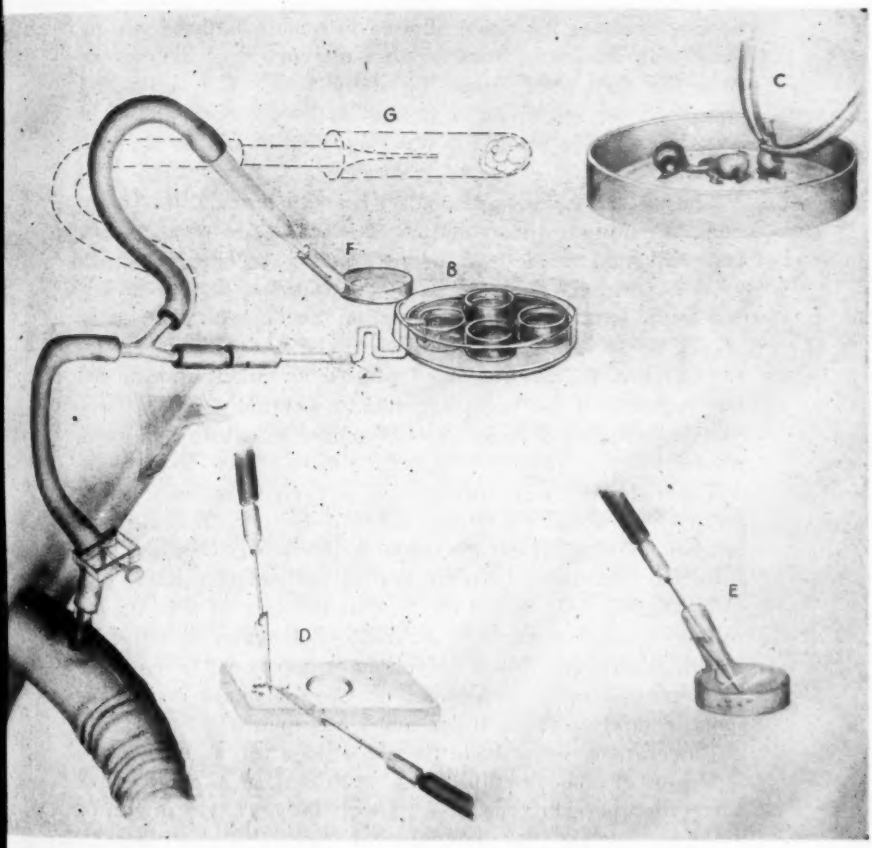

FIG. 1. Drawing of techniques used. See text for description.

bling the gas through the Tyrode's solution for about two minutes, six pieces were transferred to a Carrel flask (E) whose floor had been freshly coated with heparinized fowl plasma. Chick embryonic extract (0.15 ml.) was added, quickly mixed to form a clot, and gas was then passed into the flask for 2–3 minutes by means of a glass pipette (F). Finally the flask was tightly closed by a rubber stopper. The sterile pipette used for passing gas into the flask was stored in a glass tube (G) when not in use. No supernatant fluid was used. In no instance was the tissue allowed to remain without gassing more than 3 minutes from the time of removal of the embryo from the shell until stoppering of the flask. The air control cultures were set up in a similar manner, being placed in Tyrode's solution whose pH was controlled by means of alveolar air.

The gas mixtures contained 4% carbon dioxide, this being done to maintain a suitable pH. It is well known that hydrogen ion concentration in tissue cultures profoundly affects growth (Fischer, 1921, Earle, 1934). Carbon dioxide at 4% was found to maintain the solutions and media within the range of pH 6.4 to 7.4. These values were checked with the use of the Cambridge "Electron Ray" pH Meter. In addition to pH effects *per se*, it has been suggested by Lorente de No' (1947) that carbon dioxide may be an essential metabolite of nerve tissue.

The following concentrations of oxygen were used: 96%, 50%, 14% (alveolar air) 2% and 0%. In each case, the inert gas employed to fill out the mixture, if any was required, was nitrogen. Thus, the concentration of the second mixture was 50% O_2, 4% CO_2, and 46% N_2. In the case of 0% O_2, the mixture employed was 96% N_2 and 4% CO_2. The N_2 employed in this experiment was of exceptionally high purity: using the Haldane gasometric procedure, no O_2 was found in it. The manufacturers claimed it contained less than 0.002% O_2.

Observations were made on more than 150 flask cultures. The growth of each culture was evaluated at intervals of 24 hours for a period of one week. The quantity of cells migrating from the explant, the abundance of nerve and glial elements emerging from the explant and the amount of liquefaction (see

below) around the explant were all appraised. In many cases, in order to examine the nature of growth more closely, the preparation was stained for microscopic examination.

Results

The effects of the several concentrations of O_2 on the *in vitro* growth of the embryonic spinal cords are shown in Figures 2, 3, 4, and 5. A summary of the effects noted is given in Table I.

In 6 of 15 trials with 0% O_2 a little activity was noted; however in 9 of the 15 trials where exceptional care was taken to exclude air from the preparation, no growth whatsoever was obtained. Since nitrogen from the same tank was used in the other conditions of the experiments in which growth was seen, this lack of growth was presumably not due to toxic substances in the nitrogen, but to the lack of O_2 in the flask.

Long fibrous processes grew from most of the explants when O_2 was present. On microscopic examination with silver stains these were found to be typical nerve fibers: they showed the same characteristics as Murray and Stout (1947) report: "In general . . . the neurites, which tend to emerge first, can be distinguished by their uniformity of calibre and by their angular or tortuous course through the plasma clot . . ." The quantity of fibers growing out was directly related to the oxygen concentration: few or none at 2% O_2, many at medium O_2 concentrations but very profuse and abundant at 96% O_2, forming a dense matted network. Furthermore, it soon became obvious that migration and proliferation of cells away from the edge of the explant took place only at low O_2 concentrations; very profusely at 2% O_2, less at 14 and 50% O_2 and not at all at 96%. Some of the cells were observed to transform into typical highly phagocytic macrophages of the type described by Weiss (1944). This type of cell is believed to originate from mesenchyme. Accompanying this growth was the phenomenon of liquefaction: clear areas just at the edge of the explant suggesting digestion of the clot. This ran parallel to the quantity of cells migrating from the explant: widespread at 2% O_2, less at 14 and 50% O_2, and none at all at 96% O_2.

Discussion

The inhibition of growth by lack of O_2 which we have observed parallels Burrow's first observations for chick heart and skin (1917). In a later report, however (1921), he did observe some growth in chick heart tissue exposed to "pure" N_2. Evidently, nervous tissue requires more O_2 for growth than heart or the sarcomata.

It is apparent that cell migration, accompanied by liquefaction, is favored by a low O_2 content and inhibited by a high O_2 content. Conversely, outgrowth of nerve fibers is favored by a high O_2 content, but suppressed by a low O_2 content. The majority of the migrating cells are presumed to be glial in origin because of their form and staining reactions.

This appears to be the first instance in which the oxygen tension has been shown to control the *type* of growth to be observed from a single tissue. This phenomenon is of considerable interest in understanding the organization of cell growth. It appears to us possible that, particularly in the nervous system, growth of connective tissue elements is constantly held in check by the high O_2 tension of the nervous tissues. If this is reduced, as by a lesion which plays havoc with the vascular bed, then a small restricted focus of relative anoxia develops and the connective tissue elements therein, no longer exposed to a high O_2 tension, start to proliferate. This sort of phenomenon, indeed, may be responsible for the failure of regeneration of nerve in the central nervous system; it might be due to a failure of oxygenation of the damaged tissue, allowing overgrowth of

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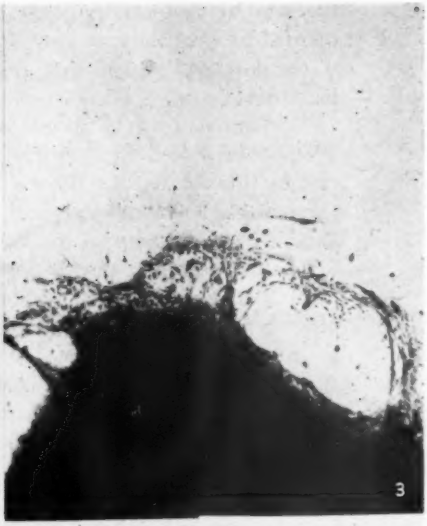
PLATE I

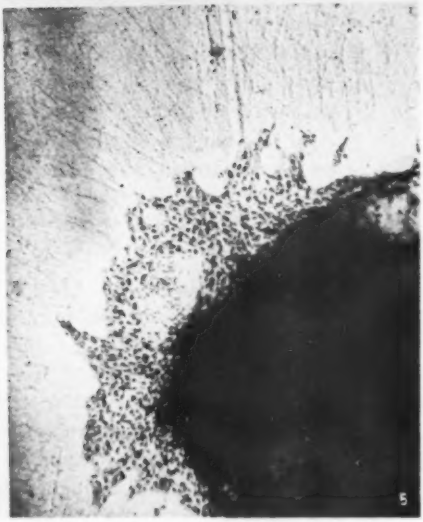
FIG. 3. Explant of 9-day chick spinal cord cultivated 48 hours at 37° C. in 96% N_2, 4% CO_2 showing no outgrowth. Bodian's silver technique. 160X.

FIG. 2. Explant of 9-day chick spinal cord cultivated 48 hours at 37° C. in 2% O_2, 94% N_2, and 4% CO_2, showing a few fibers, numerous cells and one large area of liquefaction. Bodian's silver technique. 160X.

FIG. 4. Explant of 9-day chick spinal cord cultivated 24 hours at 37° C. in 96% O_2, and 4% CO_2, showing numerous interlacing fibers and only a few cells. Bodian's silver technique. 160X.

FIG. 5. Explant of 9-day chick spinal cord cultivated 72 hours at 37° C. with alveolar air (14% O_2) showing numerous cells, fibers, and several small areas of liquefaction. Bodian's silver technique. 160X.





the site by connective tissue elements. If there were some method of providing at once for an enormous oxygen supply to the damaged region, then regeneration of true nerves might be possible.

That anoxia leads to fibrosis is a universally held view (Arey, 1936). An excellent example is the organization of a blood clot by fibroblasts. In such a locus the O_2 tension would be exceedingly low, and one can almost imagine the fibroblasts "seeking out" regions of low tensions. But then as the process of healing proceeds, the O_2 gradients change; in Burrow's concept (1924), the "degree of stagnation" changes. This allows other types of cell to grow more efficiently than fibroblasts, and so the whole character of the healing process changes. The tissue that "dominates" a mixed culture may, indeed, at times be decided by the available oxygen, as well as by pH, "wound hormones," distribution of other metabolites, etc.

The experiments of Fischer, Fogh and Jensen (1945) cast some doubt on this hypothesis. They found that tissue oxygen tensions are not responsible for the fact that the growth in tissue cultures is from the periphery only and not from its center. But since they were using embryonic chick heart fibroblasts only, and not a culture of mixed cells such as we have employed, their results should not be applied to our conditions. Also, the failure of growth in the center of an explant is undoubtedly due to other factors than O_2 tension alone.

Summary

Spinal cords from 9-day chick embryos were cultured in Carrel flasks in 96, 50, 14, 2 and 0 per cent oxygen plus 4 per cent carbon dioxide plus pure nitrogen as required. The type of growth produced differed sharply depending on the oxygen content: the outgrowth of fiber processes was stimulated by a high oxygen content but suppressed by a low oxygen content; cellular migration, accompanied by a proportionate amount of liquefaction of the medium, was stimulated by a low oxygen content (2%) but abolished by a high oxygen content. In the absence of oxygen, no growth of any type was observed.

The application of this finding to problems of regeneration of nerves and of wound healing is discussed.

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LACK OF ANTIDIABETOGENIC AND ANTIDIABETIC EFFECTS OF *TECOMA STANS* IN ALLOXAN DIABETES*

JOE B. NASH[1], C. C. ALBERS[2], JOYCE K. HOWARD, AND STERLING H. FLY, JR.[3]

Tecoma stans has been used in Mexican folk-medicine as an antidiabetic agent. Conflicting clinical and laboratory studies of its efficacy in this regard are summarized by Guerra (1946). Despite several chemical studies of *T. stans*, no active principle has been isolated; extracts apparently consist chiefly of gums and resins, with about 0.3% of a chloroform-soluble alkaloid, bignonine, and 0.05% of an ether-soluble alkaloid. Oral and parenteral administration of extracts is recognized clinically to be free of toxic sequelae. *T. stans* is known synonymously as tronadora, huachacata, retama, nixtamalxochitl, St. Nicholas' herb, *T. mollis*, *T. sorbifolia*, *Tronadora vinnadus*, *Bignonia tecomoides*, and *Stenolobium molle*. It is one of the family of Bignoniaceae.

As part of a comprehensive study of antagonists of diabetogenic effects of alloxan, it was of interest to us to include *T. stans*.

Experimental.—An aqueous extract prepared by one of us (C. C. A.), after defatting, was evaporated under vacuum to dryness. The resultant solid is poorly soluble in warm water, and was used as a suspension.

Fifteen mice were fasted 24 hours, given 1 gm./kg. of *T. stans* extract intramuscularly, and fed after 1 hour. Blood content of reducing substances was estimated by a modification of Somogyi's method (1945) on 0.02 cc. of blood from the tail, 96 hours later; it did not differ significantly from that of normal fed mice. Fifteen mice similarly treated, but with intraperitoneal injection of 1.5 *mM* of alloxan 1 hour after receiving *T. stans* extract intramuscularly, all became severely diabetic.

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Twelve mice similarly treated, but with only 0.25 m*M* of alloxan after *T. stans*, did not become diabetic; this dose of alloxan is not diabetogenic in normal mice, while 1.5 m*M* regularly produces severe diabetes or death, in our experience with several hundred mice.

Six rats, which had been rendered diabetic by intravenous injection of 0.25 m*M* of alloxan after a 40 hour fast, were given 2 intraperitoneal injections of 1 gm./kg. of *T. stans* extract, spaced 2 hours apart, 20 days after giving the alloxan. Blood content of reducing substances of the fed rats prior to the first of these *T. stans* injections ranged from 390–770 mg. % of glucose, while those taken after the second injection varied from 365–766 mg. %. The largest decrease in reducing substances in the individual rats was 25 mg. %; 3 of the 6 rats showed slight increases. Average content of reducing substances in the blood before and after treatment was identical, 488 mg. %.

The same rats were treated orally with 1 gm./kg. of *T. stans* extract on each of the successive 2 days. Blood content of reducing substances in the 5 surviving rats on the third day averaged 411 mg. % of glucose, as against 432 mg. % for these 5 rats prior to treatment.

Discussion.—Although García Colín (1926) and others have claimed that *T. stans* extracts given orally will reduce glycosuria in diabetic patients, and that hyperglycemia is reduced 2 hours after subcutaneous injection, Guerra (1946) could find no beneficial effects on diabetic patients, or any hypoglycemic response in normal rabbits or humans after oral or intramuscular injection. No glycolysis was observed in 0.1% glucose solutions incubated with *T. stans* extract at 37°C. for 24 hours. The present results with alloxan diabetes confirm Guerra's conclusions and indicate the lack of any effective antidiabetic activity in *T. stans*.

Summary

An aqueous extract of *T. stans* was found to be without antidiabetogenic activity in mice given alloxan, and without antidiabetic effect in rats with established alloxan diabetes.

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NEOMYCIN, A NEW INTESTINAL ANTISEPTIC*

EDGAR J. POTH, STANLEY M. FROMM, ROBERT I. WISE AND CHINN MIN HSIANG

The antibiotic, Neomycin, was isolated by Waksman and Lechevalier[1] from Streptomyces fradiae. Subsequently three fractions having different bacterial spectra and toxicities have been separated. The fraction studied here has the following antibacterial spectrum as compared to other antibiotics.†

Neomycin is most active in an alkaline medium, soluble in water, insoluble in organic solvents, thermostable, and bactericidal at 10 u/ml against various gram negative and gram positive bacteria. It possesses no activity against fungi.

This study is concerned with the effect of oral administration.

PROCEDURE

Neomycin was given orally to dogs and man in varying dosages on a four hour schedule throughout the 24 hour day. The dogs were maintained on a horse meat diet and the human subjects on a low residue diet.

Bacteriological studies were made to determine the total gram negative and gram positive counts, the total anaerobic organisms, the total anaerobic spores present, the coliform organisms and the alpha streptococcus fecalis daily for varying periods of Neomycin administration. Absorption and excretion of Neomycin was studied.

DOSAGE

The optimum daily oral dosage level for both man and dog was found to be 0.1 gram per kilo of body weight divided into six portions and administered on a four hour schedule. It is interesting to note that the dose for man and dog is the same,

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*From the Surgical Research Laboratory, Department of Surgery, The University of Texas Medical Branch, Galveston, Texas. Aided by a grant from The Upjohn Company, Kalamazoo, Michigan. Received for publication June 23, 1950.

†The Neomycin used in these studies was furnished by the Upjohn Company through the courtesy of Earl L. Burbidge, M. D.

TABLE 1*

Antibacterial Spectrum of Antibiotics

(expressed in micrograms per ml. required to inhibit test organisms)

	Bacitracin	*Penicillin G*	*Penicillin O*	*Aureomycin*	*Chloromycetin*	*Streptomycin*	*Neomycin*	*Terramycin*
D. pneumoniae	.13	.02	.02	.14	1.00	13.00	10.00	.13
Strep. viridans	1.00	.02	.03	.17	2.50	17.00	33.00	.17
Strep. hemolyticus	.13	.01	.02	.25	2.50	20.00	10.00	.17
Staph. albus	1.00	.03	.05	.10	2.50	.10	.01	1.00
Staph. aureus	10.00	.05	.05	.13	3.30	.05	.01	1.10
Strep. fecalis	1.00	1.00	1.70	.13	2.50	1.00	.11	1.00
B. anthracis	100.00	.01	.02	.02	1.70	.03	.02	.14
A. aerogenes	....	>100.00	>100.00	1.00	1.20	.14	.14	3.30
E. typhosa	>100.00	1.00	2.90	.67	1.20	.25	.03	2.50
E. coli	>200.00	100.00	100.00	1.00	1.70	.29	.11	2.50
Ps. aeruginosa	>200.00	>100.00	>100.00	20.00	50.00	1.70	.33	17.00
Br. abortus	....	.12	.16	.02	.50	.17	.05	.03
K. pneumoniae	....	>100.00	>100.00	.20	1.00	.13	.10	1.70
Proteus vulgaris	....	3.30	6.60	2.00	1.00	.14	.13	17.00
Salmonella schott.	>100.00	10.00	20.00	1.10	3.30	1.25	.11	4.00
M. tuberculosis (607)	....	>100.00	>100.00	.25	3.30	.50	.29	1.00
	Acute Toxicity in Mice—LD_{50} in mg. per kg.							
Intravenously	500	>1300	>1300	194	245	208	125	320
Subcutaneously	...	....	....	950	...	780	420	690

*Personal communication from Johnson, L. E. and Colingsworth, D. R.

whereas in the case of sulfasuxidine and sulfathalidine the effective dose for the dog was four times that required for man. Chart 1 shows the characteristic alteration of the intestinal bacterial flora of man.

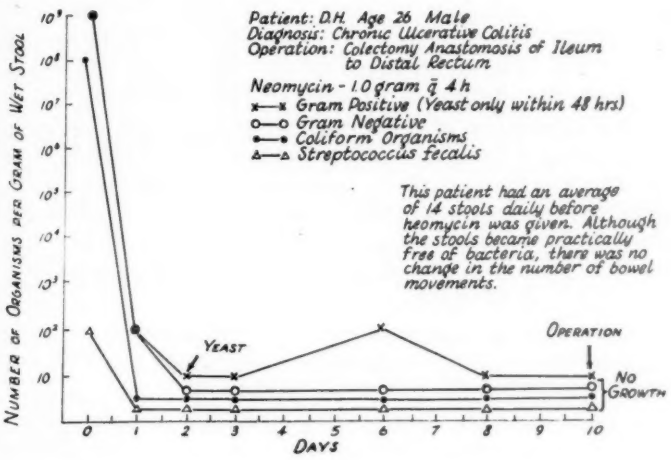
Patient: D.H. Age 26 Male
Diagnosis: Chronic Ulcerative Colitis
Operation: Colectomy Anastomosis of Ileum to Distal Rectum
Neomycin - 1.0 gram q 4 h
Gram Positive (Yeast only within 48 hrs)
Gram Negative
Coliform Organisms
Streptococcus fecalis
This patient had an average of 14 stools daily before neomycin was given. Although the stools became practically free of bacteria, there was no change in the number of bowel movements.
Number of Organisms per Gram of Wet Stool
Yeast
Operation
No Growth
Days

Chart 1.

Absorption and Excretion

The concentration of Neomycin was determined in the blood, urine and feces after the patient had been on the antibiotic for a period of three days by a bioassay method utilizing the inhibition of B. Subtilis spores in zones surrounding filter paper discs saturated with the fluid being studied.

The blood levels were extremely low with a maximum of 3 micrograms per ml. of serum. Quantitative urine assays indicate that approximately three per cent of the ingested antibiotic is excreted by the kidneys. The quantity of Neomycin in the feces is so high that the dilution factors are such as to make the actual numerical values inaccurate. But, the facts indicate that little absorption occurs from the bowel and that no appreciable amount is destroyed by passage through the gastroenteric tract.

Bowel Healing as Influenced by Neomycin

Past studies[2,3,4] have shown that alteration of the bacterial flora of the bowel has a striking influence upon the character and rate of healing at the line of anastomosis of the colon. When the flora has been reduced significantly, healing simulates primary tissue repair. The most pronounced gross and microscopic difference between control experiments and those following drug therapy occurs about the third postoperative day.

After elimination of the bacteria by the oral administration of 0.2 grams of Neomycin daily per kilo of body weight to dogs for three days as previously outlined, the midcolon was transected and then immediately anastomosed by a single, continuous, through-and-through, inverting suture of 00 chromic catgut. The control animals were similarly treated except that Neomycin was not given.

On the third postoperative day the animals were sacrificed, the anastomosis recovered, examined, photographed in color on mucosal surface, and microscopic sections taken.

Gross examination of the control specimens shows edema of the mucosa extending a distance of 3 to 5 cm. on either side of the anastomosis. There is always gross necrosis of the cut edges of the bowel. Microscopic examination of the tissue at the line of suture shows extensive edema, massive polymorphonuclear infiltration and areas of tissue necrosis. There has been no ingrowth of vascular capillaries. Fibroplasia has been practically completely suppressed.

Gross examination of the specimens following elimination of the bacteria shows edema of the mucosa extending not more than 1.0 cm. from the line of anastomosis. Necrosis of the cut edges of the bowel does not occur. Microscopic examination of the tissue at the line of suture shows some edema, moderate polymorphonuclear infiltration, ingrowth of vascular capillaries, and extensive fibroplasia. There is no evidence of tissue necrosis. The process is that of primary healing.

On those occasions where the bacteria have not been eliminated by the administration of Neomycin, the healing process simulates that of the control experiments. It appears likely then that it is not the presence of the Neomycin, but rather the

absence of the bacteria which makes the difference in the healing process.

It should again be emphasized that no new antibiotic or chemotherapeutic agent should be used in human treatment until after the effect of these substances in contact with the injured tissues has been determined experimentally. It is not inconceivable that any one of these substances might strongly suppress healing and so invite catastrophe.

These studies demonstrate quite conclusively that healing is favored and accelerated when the bacterial flora is significantly altered by the oral administration of Neomycin.

Mouse Test for Alteration of Feces

One cc. of a 10% suspension of human feces injected intraperitoneally into a 20 gram mouse results in the death of 100% of the animals in 12 to 24 hours. Immediately after the elimination of the bacterial flora by the administration of Neomycin, 100% of mice injected with the same quantity of the altered feces survive.

If Neomycin has been administered, but some bacteria persist, the death rate will vary depending on the degree of bacterial insult. This test appears to be an excellent procedure and has many obvious applications.

Clinical Application

Neomycin has been administered to 26 patients during the past four months. The routine preoperative preparation has consisted of a purge of castor oil, unless it was contraindicated, Neomycin 1.0 gram q 4 h. Usually three days medication is sufficient to eliminate the usual bacterial inhabitants of the bowel and empty the bowel of feces and gas.

The patient usually has four to eight bowel movements daily. These movements may be thin and watery, but they are not copious and are not accompanied by gripping pains. They do not tend to dehydrate the patient. During the preoperative period a low residue diet is permitted. The stools become odorless, semisolid to fluid in consistency, and greenish in color. One cc. of a 10% suspension of these feces injected into the peritoneal cavity of a mouse causes no demonstrable reaction.

All of these patients have been subjected to operations on the colon. There has been no instance of peritonitis or fecal fistulae postoperatively. Untoward toxic symptoms have not occurred. The postoperative temperature elevations have been slight and ordinarily did not extend beyond the first postoperative day.

Effect of Oral Neomycin on the Clotting Mechanism

Careful bleeding times, clotting times and prothrombin times were determined on ten individuals. One patient was maintained on Neomycin for five weeks. During this period the stools were free of bacteria. Yeast was present. There was no change in bleeding, clotting or prothrombin times. In no instance has there been any demonstrable change in the clotting mechanism. There has been no evidence of increased bleeding tendency.

Discussion

The limited degree of absorption of the fraction of Neomycin here studied precludes the likelihood of severe toxicity. This supposition has been verified by the absence of toxic reaction in the patients treated.

It is interesting to note that this antibiotic appears to be most effective when there are frequent, watery stools. This observation is just the reverse of that noted in the case of sulfasuxidine and sulfathalidine. There effectiveness was greatly lowered in the presence of diarrhea, and the use of purgatives was contraindicated.

Neomycin is only slightly less effective in dog than in man. These findings are again at variance with the observations on the sulfonamides wherein the effective dosage for dogs was four times that required for man.

The ideal intestinal antiseptic should have the following properties:

1. Low toxicity for the patient.
2. A broad bacterial spectrum.
3. Prevents development or overgrowth of resistant bacterial variants.
4. Rapid activity.

5. Limited absorption from the gastroenteric tract.
6. Permits adequate food and fluid intake.
7. Assists in mechanical cleansing of the bowel without causing dehydration.
8. Will not cause gastroenteritis.
9. Promotes tissue healing.
10. Low dosage.
11. Soluble in water.
12. Palatable.

Our studies to date indicate that Neomycin fulfills all of these requirements.

Summary

1. A fraction of Neomycin isolated from Streptomyces fradiae has been studied following oral administration.
2. It is poorly absorbed from the gastroenteric tract.
3. It rapidly eliminates the usual bacterial inhabitants from the bowel.
4. It is nontoxic.
5. It effects emptying and decompression of the bowel.
6. It promotes tissue healing.
7. It appears to be an excellent intestinal antiseptic and adjuvant to abdominal surgery.

This 26 year old male has had chronic ulcerative colitis in severe form for three years. A goodly portion of this time has been spent in hospitals following acute exacerbations of the disease. He has been treated with all of the sulfonamides and antibiotics with only temporary relief. Neomycin caused an abrupt elimination of bacteria from bowel, but there was no change in the symptoms and diarrhea. X-rays showed extensive anatomical changes in the entire colon with loss of contractility. Sigmoidoscopic examinations showed the rectum to be relatively normal. At operation the distal ileum was found to be involved in the inflammatory process. The distal 30 cm. of ileum and colon were resected and the proximal stump of the ileum anastomosed to the rectum below the level of the pelvic peritoneal reflection employing the lateral-end to end technique.[5] The immediate postoperative course was entirely smooth with a maximum of 1° F. rise in temperature for one day only.

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PROCEEDINGS OF THE TEXAS BRANCH OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

Spring Meeting, Dallas, April, 1950

Titles

Some Metabolic Characteristics of Hydrocarbon Oxidizing Bacteria. J. B. Davis and H. H. Chase. Field Research Laboratories Magnolia Petroleum Company, Dallas.

Development of Peptone Toxicity with Aging. L. Rode, V. T. Schuhardt and Glenda Oglesby. Dept. of Bacteriology, The University of Texas, Austin.

Studies on the Growth Requirements of *Streptobacillus moniliformis*. Morris Dumoff and Carl E. Duffy. Dept. of Bacteriology and Parasitology, University of Arkansas School of Medicine, Little Rock.

Comparison of Heat and Chemical Resistance in Bacterial Spores. F. Davis. Dept. of Bacteriology, The University of Texas, Austin.

In vitro Titrations of Mycomycin against *Mycobacterium tuberculosis*. Martha Wilkerson and D. E. Jenkins. Dept. of Medicine, Baylor University College of Medicine, Houston.

Evidence of Seasonal Variation in the Bacterial Flora of the Upper Respiratory Tract. A. M. Lonergan, C. D. Dukes, Allergy Research Foundation and Dept. of Bacteriology, Baylor College of Dentistry, Dallas.

Abstracts

Observations on Bacterial Spore Formation in Synthetic Media. E. B. Blair. Department of Bacteriology, The University of Texas, Austin, Texas.

Quantitative observations and quantitative counts on *Clostridium botulinum* grown in modifications of the synthetic medium of Roessler and Brewer indicate that sporulation by this organism is sharply stimulated by either glycine or increased levels of arginine, and to a slight extent by either ornithine, glutamic acid, asparagine, sodium butyrate, creatine, or proline. No stimulatory effect has been observed for cystine, norleucine, sodium formate, sodium glyceraldehyde phosphate, choline, or serine. The omission of methionine appears to suppress sporulation, and the addition of glycine, choline, or cystine does not overcome the effect. Glucose favors growth but inhibits spore formation. Phenylalanine, tested in complex media, stimulates growth but represses spore formation.

The Action of Microorganisms of Marine Origin on Trimethylamine Oxide and Related Compounds. L. Leon Campbell, Jr. Department of Bacteriology, The University of Texas, Austin, Texas.

Seven strains of hitherto undescribed species of Achromobacter have been studied for their action on trimethylamine oxide, choline, acetyl choline and betaine. Four of the seven strains produced trimethylamine from the oxide. Using the Thunberg technique it was found that the reduction of the oxide, in presence of various hydrogen donators, namely glucose, pyruvate, lactate, and acetate, is a linear function of time. It was further shown that these reducing strains possess a heat labile enzyme which activates trimethylamine oxide, rendering it susceptible to the reducing systems of the cell.

Two of the seven strains produced trimethylamine from choline and acetylcholine, while none of the strains produced the amine from betaine. It was found that organic acid accompanied the production of trimethylamine in unbuffered choline broth, as the pH fell from 7.0 to 4.0 in 18 hours. The effect of available carbohydrate on choline decomposition revealed that at low levels of glucose, the production of trimethylamine was decreased, with 1% glucose showing a complete inhibition of trimethylamine production.

This suggests that these organisms may decompose choline by utilization of the alcoholic side chain as a source of energy, setting trimethylamine free as a result of the oxidative cleavage of the carbon-nitrogen bond. Preliminary tests indicate the presence of acetate in the culture filtrates, although this has not been definitely established.

Growth of Bacteria in Media Containing Technical Pentaerythritol. G. A. Emerson, P. L. Ewing, Bernice M. Doucet and Benjy F. Brooks. The University of Texas Medical Branch, Galveston, Texas.

Pentaerythritol (tetramethylolmethane) (*I*), first prepared by Tollens and Wigand (Ann., *265*: 316, 1891) is now used in

the paint industry because of its superiority to glycerol in preparing drying oils, as a technical mixture (Pentek) of about 15% dipentaerythritol and 85% *I*. *I* is tolerated by animals in the maximal doses practicable to give, is eliminated unchanged in the urine and is not irritant to the skin as a saturated (approx. 10%) aqueous solution. We are grateful to the Heyden Chemical Corporation, New York City, for this information and for a generous supply of pentek.

I was examined as a source of C to support bacterial growth, and for a possible bacteriostatic effect through competition with utilization of other carbohydrates. In a synthetic medium lacking carbohydrate, similar to that of Porter and Pelczar (J. Bact., *41:* 173, 1941) but with $< \frac{1}{2}$ the amounts of amino acids recommended and fortified with minute amounts of additional vitamins, purines and pryimidines, no growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus enteritidis* or *Proteus* sp. occurred with or without addition of 1 or 2% *I*, while good growth occurred with addition of 1% dextrose. In broth cultures, *S. aureus*, *E. coli*, *P. aeruginosa*, *Streptococcus fecalis*, *Aerobacter aerogenes*, and *Alcaligenes fecalis* grew well in the presence of 0.1–500 mg. % *I*. The above organisms and *Eberthella typhosa* (2 strains), *Salmonella schottmuelleri*, *S. paratyphi*, *S. Derby*, *Shigella paradysenteriae alkalescens*, *Sh. paradysenteriae* Flexner, *Sh. paradysenteriae* Sonne, *Proteus morgani*, *P. vulgaris*, and additional strains of *S. aureus* showed growth on nutrient agar containing 0.8% *I* $\leqq$ that of controls without *I*. Group A *S. hemolyticus* grew abundantly on blood agar containing 0.8% *I*, also with an apparent stimulation of growth beyond that of the controls. We wish to express our thanks to Professor William B. Sharp for affording us the facilities of his laboratory.

These results imply that while most if not all bacteria lack the ability to utilize *I*, no bacteriostasis can be expected at concentrations attainable for appreciable periods in body fluids, despite the high tolerance of animals to *I*.

The Effect of Urea Derivatives and Sodium Sulfite on Anti-Rh Antibodies. Ruth Guy, Sol Haberman, and J. M. Hill. Graduate Research Institute, William Buchanan Blood Center, Baylor University Hospital, Dallas, Texas.

Anti-Rh serum was treated with urea and some of its derivatives (thiourear, and guanidine hydrochloride) and with sodium sulfite. The action of these chemicals demonstrated some of the chemical properties of the three orders of antibody activity. Urea inactivated the saline agglutinin and produced a decrease in the cryptagglutinoid titer to about one-half its original strength. The action of urea was reversible by the use of urease or by dialysis. Thiourea destroyed the saline agglutinin and greatly decreased the cryptagglutinoid titer with no observable change in the agglutinoid (blocker). Guanidine hydrochloride in 5M concentration attacked all three orders of antibody activity. The sodium sulfite selectively inactivated the agglutinnoid. The sodium sulfite reaction was reversible with potassium bromate. These experiments give further evidence of the independence of agglutinins, agglutinoids, and cryptagglutinoids as definite chemical entities. In addition, such experiments give some insight as to the chemical composition of immune globulins.

The Occurrence of Types of Influenza in 1950. Dwight M. Kuhns, Ralph O. Anslow, Evelyn Maraist and Dorothy Shelton, U.S. Air Force Laboratories, Randolph Field, Texas.

Beginning in January 1950, clinical cases of "old fashioned la grippe" or influenza began to appear both in the military and civilian population. The clinical picture was characterized by sudden onset of headache, malaise, severe aching all over the body, especially affecting the back. Also, along with this there was some sore throat and coryza. The temperature was elevated and sometimes was as high as 103–104° F. It is realized that it is difficult to diagnose influenza by clinical observation alone, therefore, early in the season arrangements were made with all dispensaries and hospitals in our area to submit paired specimens of sera occurring early in the disease (about one week to ten days later) to this laboratory for agglutination-inhibition tests with influenza A, A', and B Types.

So far this year we have had a total of 212 paired sera submitted and among these were the following types:

INFLUENZA AGGLUTINATION-INHIBITION TESTS

February-March, 1950

Total paired specimens received	240
Of these, positive to A, A', or B	64
Positive to A alone	4
Positive to A' alone	21
Positive to B alone	2
Positive to A and A'	37

AGGLUTINATION INHIBITION RESULTS WITH NEWLY ISOLATED STRAIN, RANDOLPH AIR FORCE BASE RB-1-50

Using 100 paired sera

34 pair positive for A, A', and RB-1-50

5 pair positive for RB-1-50 only

The above typing of sera were all confirmed by the Army Medical Department Research and Graduate School.

It would appear as far as types of influenza are concerned that a vaccine prepared from influenza type A, A', and the Fourth Army Area Medical Laboratory newly isolated strain RB-1-50 should have protected against the majority of cases of influenza that occurred in this area this year.

Due to the occurrence of other respiratory diseases it has been difficult to say whether we have had an epidemic of influenza, but we do feel that a considerable number of the respiratory disease illnesses were due to the above named types of influenza.

The Sensitivity of *Gaffkya Tetragena* Strains to Penicillin and Streptomycin. Frank S. Mock and E. Staten Wynne. Department of Plant Sciences, University of Oklahoma, Norman, Oklahoma.

By means of the broth serial dilution method, all of six strains of *Gaffkya tetragena* were inhibited by one unit per ml or less of penicillin, and by .06 unit per ml or less of streptomycin. An agar dilution plate method showed somewhat lower susceptibility, with inhibitory concentrations ranging up to two units per ml of penicillin and one unit per ml of streptomycin.

Five of the six strains became resistant to 1000 units per ml of streptomycin within five transfers on agar containing graded concentrations of the abtibiotic. The streptomycin-resistant variants showed no increased resistance to penicillin. Furthermore, direct attempts to secure increased penicillin resistance by a technique similar to that used with streptomycin were unsuccessful.

Effects of Antibiotics and Other Agents on Development of *Mycobacterium Tuberculosis Hominis* in the Chick Embryo. Joe B. Nash, William M. Gambrell, Bernice M. Doucet and G. A. Emerson. The University of Texas Medical Branch, Galveston, Texas.

Goodpasture and Anderson (Am. J. Path., *13:* 149, *1937*), *Costil and Bloch* (Compt. rend. Soc. Biol., *128:* 40, *1938*), *Emmart and Smith* (Publ. Health Repts., *56:* 1277, *1941*), *Moore* (J. Bact., *41:* 786, *1941;* Am. J. Path., *18:* 827, *1942*) and *Canat and Opie* (Am. J. Path., *19:* 385, *1943*) have described growth of mycobacteria on the chorioallantoic membrane of the chick embryo, with invasion of the tissues. *Lee* (Proc. Soc. Exptl. Biol. Med., *61:* 143, *1946;* Am. Rev. Tuberc., *55:* 262, *1947;* Ann. N. Y. Acad. Sci., *52:* 692, *1949*) has developed an intravenous inoculation technique for 11–12 day old chick embryos which is claimed to give a more uniform degree of infection than yolk-sac inoculation and to permit treatment with chemotherapeutic agents at a site remote from the site of inoculation. We have found that 7–8 day old chick embryos inoculated with massive doses of *M. tuberculosis hominis* via the yolk-sac are regularly infected and show a high mortality before time of hatching. In view of the greater skill needed, the considerable operative mortality and the decreased time available for treatment in Lee's procedure, we believe that the simpler procedure of yolk-sac inoculation, using larger numbers of eggs with adequate controls for each series, amply fulfills the aim of providing an inexpensive in vivo screening test for chemotherapeutic agents. The yolk-sac inoculation technique is inferior to Lee's method chiefly in differentiating between activities of relatively ineffective agents, but such minor differences are of questionable significance. Application of the yolk-

sac inoculation technique to study of the potentiation of streptomycin by iodides, and to a comparative study of efficacy of the diethylethanolamine ester of 3-phenyl-salicylate, neomycin and streptomycin are discussed.

Further Observations on Viruses of the Coxsackie Group Recovered from Human Stools. Joyce W. Rowen and J. V. Irons. Texas State Department of Health, Austin, Texas.

In 1947, Dalldorf and Sickles reported the recovery of a virus from human stools which was characterized by its resistance to ether and pathogenicity for infant mice. This virus showed marked tropism for muscle tissue and the resulting myositis was usually sufficiently extensive that it resulted in paralysis and death of the mice.

This is a report on the recovery of two viruses. The first strain, B. J., was found in the stool of a paralyzed child who became ill in August, 1949, in San Antonio. The second strain, B. G., was found in the stool of a child who became ill at Abilene late in November, 1949. The results of our serum-virus neutralization tests left no doubt these two viruses were members of the "Coxsackie group" and were, in fact, of two serologically distinct types, corresponding to T. T. (type 1) and J. O1. (type 3), as designated by Dalldorf.

The Texas 1–2 strain of Melnick, originally found in the lower Rio Grande Valley, and later by Sulkin, Manire and Farmer in the northeast Texas, according to Dalldorf, differs serologically from the strains which he has designated 1, 2 and 3. Thus, of at least 5 serologically distinct types of the Coxsackie virus so far designated, three of these types have been found in Texas, from various parts of the state.

The Influence of Vitamin B_{12} on Experimental Viral Infections. S. Edward Sulkin, G. P. Manire and Rae Allen. Department of Bacteriology, Southwestern Medical School of The University of Texas, Dallas, Texas.

These studies are concerned with the effect of vitamin B_{12} on the course and outcome of experimental infections with the viruses of St. Louis encephalitis and poliomyelitis (Lansing).

When the experiment with the Lansing strain of poliomyelitis virus was terminated 30 days following virus inoculation, there was no significant difference in the mortality rates in the vitamin B_{12}-treated animals as compared with the controls. Vitamin B_{12}, however, did influence the course of the experimental infection produced with the St. Louis encephalitis virus. Animals receiving vitamin B_{12} before or after virus inoculation showed increased susceptibility to this experimental infection. This observation was found to be reproducible.

In another experiment each animal in two groups of 40 mice each was inoculated intracerebrally with approximately one LD_{50} dose of St. Louis encephalitis virus. One group served as controls for the second which received vitamin B_{12}. The effect of the B_{12} on the cause of this experimental infection became evident early in the experiment. By the sixth day, 60% of the B_{12} treated animals had died while only 35% of the controls succumbed to the infection. When the experiment was terminated 15 days after the virus inoculation, 82.5% of the animals receiving B_{12} had died as compared with only 55% of the controls. Thus, it is evident from these data that vitamin B_{12} modified the course and outcome of the experimental infection with the St. Louis encephalitis virus.

Interference Between Poliomyelitis Virus (Lansing) and the Coxsackie Virus. S. Edward Sulkin and G. P. Manire. Department of Bacteriology, Southwestern Medical School of The University of Texas, Dallas, Texas.

It has been repeatedly observed that the carrier state with the Coxsackie and poliomyelitis viruses may exist simultaneously. Although, under certain conditions. two viruses may propagate simultaneously in one host and in the same organ or tissue, there remains the possibility that interference between the agents may occur. Studies were undertaken to determine whether the Coxsackie virus would influence the disease produced with the Lansing poliomyelitis virus in three week old mice, and whether the Lansing virus would influence experimental infection produced with the Coxsackie virus in three day old mice.

The Lansing poliomyelitis virus and the Dallas-M. B. Coxsackie virus, hereafter referred to as "C" virus, were used. The first series of experiments indicate that "C" virus failed to alter significantly the course of the experimental Lansing poliomyelitis infection.

Injections of the Lansing poliomyelitis virus into three day old white Swiss mice significantly influenced the course and outcome of infections with "C" virus. By the fourth day, only 34.6% and 37.9% of two groups of animals receiving inoculations of Lansing virus in addition to "C" virus had succumbed as compared with 77.8% of the animals receiving "C" virus alone. When the experiment was terminated, all of the controls receiving "C" virus alone had died while only 84.7% and 72.4%, respectively, of the animals receiving interfering injections of Lansing virus had died.

Antibacterial Factor in Ticks. II. The Role of Blood of Host Animals. Dorothy M. Whitney, Ludwik Anigstein, and Don W. Micks, Department of Preventive Medicine and Public Health, The University of Texas Medical Branch, Galveston, Texas.

It was shown previously that extracts of trypsin digested ticks are provided with antibacterial properties. This activity was of higher intensity in blood engorged ticks as compared with unfed specimens; therefore, it was of interest to determine whether this enhacing factor is present in the blood of the host animal. Lyophilized clots from blood of man, cattle, dogs, rabbits, chickens were incubated with trypsin for 5 hours at 37 C in 0.1 M phosphate buffer (pH 7.8). The digested products were tested for antibacterial activity against *Bacillus subtilis* and related spore-bearing bacilli. Complete or partial inhibition of growth in all members of this group was demonstrated by serial dilution on agar plates (from 1:100 up to 1:4,800). The range of antibacterial activity includes *Escherichia coli, Salmonella typhimurium, Sarcina lutea, Staphllococcus albus and S. aureus.* The results are based on 26 samples of blood of various origin. No antibacterial activity was shown either in the serum or in the nondigested portion of the blood under described conditions.

Effect of Penicillin on the Germination of Spores of Certain Clostridium Species. E. Staten Wynne and Knox Harrell. Department of Plant Sciences, University of Oklahoma, Norman, Oklahoma.

In preliminary experiments one unit of penicillin per ml prevented the appearance of turbidity at an inoculum level of approximately 50 spores per ml with *Clostridium botulinum* 62A, *Clostridium perfringens*, and putrefactive anaerobe No. 3679. This effect could be due to (1) an inhibition of germination, (2) a sporocidal effect, or (3) an inhibition of vegetative cell development.

By means of the quantitative technique of Wynne and Foster (*J. Bact.* 55:61–67, 1948) it was shown that even 1000 units of penicillin per ml was without effect on germination. Similarly, a sporocidal action could not be demonstrated.

That the site of action of penicillin in these species is actually the multiplication of vegetative cells resulting from the germination of spores was supported by direct tests of the antibiotic on vegetative cells. With *Clostridium botulinum* 62A, 0.1 unit of penicillin per ml prevented development of turbidity from an inoculum of around 100 vegetative cells per ml during an observation period of three weeks.

"A Method for Collection of Bacteria, Actinomycetes and Filamentous Fungi from Air." Roland B. Mitchell, Harry W. Dorris, Jr., Durward E. Timmons, and Dorothy T. Anderson. U.S. Air Force Laboratories, Randolph Field, Texas.

A water-soluble dry-foam gelatin material has been developed which can be sterilized and possesses the necessary tensile strength for preparation of efficient filters to be employed as collection devices for air-borne microorganisms present in large volumes of extramural and intramural air.

The use of this soluble gelatin foam as a filter eliminated the problem of recovering microorganisms from a collection device. An exposed gelatin-foam filter dissolves when placed in water, frees the collected bacteria, actinomycetes and fungi and leaves them suspended in the liquid. This suspension can be employed for making quantitative and qualitative analyses of the microorganisms content of a definite volume of air filtered through the soluble dry-foam gelatin filter.

OBSERVATIONS ON THE EFFECT OF CERTAIN OF THE ANTIMALARIAL DRUGS ON ERYTHROCYTES

R. H. Rigdon and Dorothy Breslin*

Much has been written with regard to the mechanism of the effects of antimalarial drugs on plasmodia; however, no single theory at this time is widely accepted. Many think that the various drugs have a direct plasmodicidal effect, while others believe that the effect is indirect and results from the stimulation of the defense mechanism within the host (Sollmann, 1942; Field, 1938; Black, 1946). Christophers and Fulton (1938) say, "It is thought as a result of experiments upon the inhibitive action of drugs that those which affect the red cells in a marked degree, *e.g.*, methylene blue, are particularly effective against parasite substance. This would suggest that the parasite is very vulnerable through the red cell, and it seems not improbable that oxygen is normally obtained through the oxyhemoglobin of the cell." The significance of the erythrocyte-plasmodium relationship is emphasized by the work of Wendel (Wendel, 1943). He thinks that the parasite is probably dependent upon the host both for its metabolic processes and the disposal of end products of its metabolism. If such products are allowed to accumulate, they may be capable of destroying parasites.

Recent studies in this laboratory on the effect of phenylhydrazine hydrochloride on *P. knowlesi* infection in *M. mullata* monkeys lend support to the possible significance of the erythrocyte-plasmodium relationship as a possible significant mechanism in the inhibition of a malarial infection (Rigdon and associates, to be published). In these studies it has been shown that the oral administration of three doses of 150 milligrams or less of phenylhydrazine hydrochloride in 95 per cent alcohol always inhibits an acute *P. knowlesi* infection in monkeys. Animals thus treated are resistant to subsequent inoculation of *P. knowlesi* and some of the monkeys fail to develop spontaneously this

*From the Laboratory of Experimental Pathology, The University of Texas Medical Branch, Galveston, Texas. Submitted for publication June 14, 1950.

infection following a splenectomy. These studies would suggest that the inhibition in the degree of parasitemia results primarily from the effect that phenylhydrazine produces upon the erythrocyte. It is well known that phenylhydrazine will produce methemoglobin in man and certain laboratory animals. Although there may be a variation of opinion as to the mechanism by which phenylhydrazine acts, all investigators do agree that the erythrocyte is affected (Webster, 1949). Webster (1949) recently reviewed the problem of the changes that occur in erythrocytes following the administration of certain chemical agents, one of which was phenylhydrazine. Goodman and Gilman (1941) suggest that the action of phenylhydrazine on erythrocytes is to split the hemoglobin into hemin and globin and that at least a portion of the remaining hemoglobin is catalyzed by the globin to form methemoglobin and perhaps other unidentified substances. Heubner (1941) regards the action as an opening of the tetrapyrrol ring of hemoglobin, the toxic material or some derivative thereof being able to modify the globin which is precipitated as a Heinz body and acquires an affinity for basic stain.

In further support of the observation that erythrocytes are affected by phenylhydrazine is the observation of Morawitz and Pratt (1908) that erythrocytes from animals poisoned by this drug are more resistant than normal as measured with hypotonic salt solutions and hemolytic agents. The erythrocytes from monkeys treated with phenylhydrazine are not hemolyzed in distilled water.

It is of interest to review the action of several of the antimalarial drugs on erythrocytes and to find that treated animals frequently show methemoglobin, hemoglobinemia and peculiar bodies within the erythrocytes that suggest that some change has been produced in the red cell by the therapeutic agent.

Quinine

Many explanations have been offered to explain the mechanism of the effect of quinine in malaria. However, at this time there appears to be no uniformity of opinion as to its action. Sollmann (1942) has suggested that quinine might stimulate the defense mechanisms of the body. Russell and associates

(1946) suggest that this drug may make the erythrocyte distasteful for habitation by parasites. Wiselogle (1946) says, "It appears likely then that the antimalarial activity of cinchonine is derived from the ability of cinchonine rather than an active intermediate, to participate in an essential biological system of the parasite or a shared biological system in the parasitized erythrocyte." Recently Mackerras and Ercole (1949) concluded that quinine appears to act by inhibiting the metabolism of hemoglobin. It has no action on parasites whose growth is independent of this protein nor on gametocytes which have already completed their growth. Quinine has been found within erythrocytes (Kelsey and associates, 1943; Glasko, 1943) and methemoglobin in fatal cases of quinine poisoning (Varton and Discombe, 1940). The occurrence of hemolytic anemia and hemoglobinemia following quinine therapy lends support to the idea that the content of the erythrocytes may be affected by this drug, and such a change may be detrimental to the development of some forms of the parasite. It is well established that quinine does inhibit the oxygen consumption of malaria parasites (Silverman and associates, 1944). If quinine inhibits the metabolism of hemoglobin as suggested by Mackerras and Ercole (1949), the parasite may find the hemoglobin of the erythrocyte either changed or the oxyhemoglobin inadequate for its normal propagation.

Pamaquine

Pamaquine is recognized to be a very valuable drug in relapsing *vivax* malaria when used concurrently with quinine (Berliner et al., 1948). It is also a potentially dangerous drug (Roskott and Seno, 1928). Acute hemolytic anemia constitutes the most serious hazard incidental to the administration of pamaquine (Cordes, 1928). The adverse reactions due to pamaquine, which are referable to the blood, include the production of methemoglobin, the occurrence of hemolytic anemia and neutrophilic granulocytopenia (Earle and associates, 1948). The degree of methemoglobinemia varies in different animals following the administration of pamaquine. It is considerably less intense in the monkey than in the dog (Wiselogle, 1946). Wiselogle (1946) says, "A clue to the nature of the products

formed by the metabolism of pamaquine and related animoquinolines is found in the observation that methemoglobinemia uniformily results from their administration to animals."

Manson-Bahr (1927) reported instances of Blackwater Fever accompanying plasmoquine (pamaquine) therapy with hemolytic icterus and the destruction of one and a half million red corpuscles per centimeter of blood. Eiselsberg (1927) reported a case in which a man who did not have malaria was accidentally treated with plasmoquine (pamaquine). He developed a severe anemia with an accompanying hemoglobinuria. Methemoglobin in the urine was spectroscopically established. It was observed that the amount of plasmoquine given this patient was less than the accepted daily dose. Orachowatz (1928) investigated two cases of pamaquine cyanosis and found "the oxygen capacity of the blood was reduced to two-thirds of the normal as the result of the conversion of hemoglobin into methemoglobin." Fischer and Weise (1927) accredited the cyanosis following pamaquine therapy to the methemoglobinemia and made spectroscopic examinations of the blood. They discovered that "of twenty-six patients tested daily for some weeks, methemoglobinemia was absent from only one, in the others it ranges from 4 to 26 percent." The first appearance of methemoglobinemia was twenty-four hours after the first plasmochin injection and the last evidence was twelve days after the cessation of administration.

Palma (1928), when treating a case of malaria with plasmochin (plasmaquine), found that "when cyanosis was at its height there appeared in the red corpuscles rounded bodies staining dark with the May-Grunwald-Giemsa stain and which are identified as Ehrlich-Heinz bodies or the capsule of the endoplasm of the corpuscle."

It is obvious from the above cited observations that pamaquine frequently affects the red cell. The presence of Heinz-Ehrlich bodies indicates a marked disturbance in the hemoglobin of the erythrocytes. Could it be that this disturbed metabolic process is detrimental to the growth and multiplication of certain forms of the malarial parasite?

Pentaquine

Pentaquine is a very effective antimalarial drug against *Plasmodium vivax*. Its toxic effects resemble those of pamaquine although to a lesser degree. Loeb (1946) determined the toxicity of this drug for various experimental animals. According to him, pentaquine produces methemoglobinemia in the dog but fails to produce it in the monkey. Loeb (1946) also administered pentaquine in doses of 60 milligrams to 171 white human volunteers and methemoglobinemia was demonstrated in 44. Alving et al. (1948) treated 74 patients with 60 milligrams of pentaquine and only 9 failed to show evidence of methemoglobinemia in access of the mean value. They observed that "the production of methemoglobin was common, and, in effect, further reduced the amount of available oxygen-carrying hemoglobin." Craige and associates (1948) have studied the toxicity of pentaquine. They found that each of a group of 20 volunteers had methemoglobinemia when one dose of 180 milligrams was given daily for eight days. A very high degree of methemoglobinemia also was observed in the group receiving 120 millograms per day. In the latter group, a loss of total hemoglobin combined with conversion of hemoglobin to methemoglobin produced a marked reduction in the blood pigment available for oxygen transport. Rarely pentaquine produces an acute hemolytic anemia (Boyd, 1949).

Sulfanilamide Compounds

Although the sulfa compounds are useless in the treatment of malarial infections in man, sulfanilamide has been shown to be a very potent drug in the treatment of *P. knowlesi* infection in the monkey (Coggeshall, 1938). The effect of this group of drugs on the erythrocyte, therefore, is significant in this problem. The ability of sulfanilamide to convert hemoglobin into methemoglobin has been known for many years (Wendel, 1939; Bensley and Ross, 1937; Harris and Michel, 1939; Colebrook and Purdie, 1937). Mull and Smith (1938) found, "In one case at least, there was not only a cyanosis, which is frequently observed, but an actual lowering of the capacity of the blood for oxygen." Hartman and associates (1938) have said,

"Reasonably close agreement exists between the direct spectroscopic determination of methemoglobin and the determination of non-oxygen carrying hemoglobin." Moeschlin (1940) discovered Heinz-Ehrlich bodies in the blood of many persons treated with sulfapyridine. Richardson (1940 and 1941) produced Heinz bodies in mice by giving sulfanilamide, sulfathiazole, sulfapyridine, and sulfanilylguanidine. He also demonstrated that when the largest number of Heinz bodies and the greatest concentration of sulfhemoglobin were present the oxygen capacity of the blood was lowered.

It has been shown that sulfanilamide produces a complete cure of either acute or chronic *P. knowlesi* infections in the monkey (Coggeshall, 1938). In this type of malaria, Coggeshall and Maier (1941) observed that mature schizonts required more oxygen than ring forms. These investigators also demonstrated that sulfanilamide produced an inhibitory effect on the respiration of the parasites in all experiments in which mature parasites were used, but it was without effect in those experiments where ring forms were present. Coggeshall (1940) determined the respiration requirements of *P. knowlesi* and *P. inui* infections in *M. rhesus* monkeys and concluded, "The oxygen consumption for *P. knowlesi* was approximately six times greater than for the same number of *P. inui* parasites." Then, while using mixed infections of *P. knowlesi* and *P. inui*, he showed that sulfanilamide completely eradicated the *P. knowlesi* infection but left the animal with a chronic *P. inui* infection. This observation would suggest that parasites requiring the greatest amount of oxygen are most severely injured by sulfanilamide.

Chaudhuri (1942) used normal monkeys to determine the effect of sulfanilamides on their hemopoietic systems. He found, "The earliest signs of anemia, as manifested by a fall of hemoglobin and total red blood cells, appeared between 24 and 72 hours after the onset of treatment by both prontosil and M&B 693. The maximum anemia in all cases occurred within 7 to 10 days." Chaudhuri (1942) also stated that "the experimental evidence, therefore, tends to show that the anemia caused by administration of sulfanilamide drugs is hemolytic in origin."

Tyrothricin

Plasmodium gallinaceum infection in chicks has been treated with tyrothricin by Taliaferro et al. (1944). This study is particularly interesting from the standpoint of the effect of tyrothricin on the red blood cells. They found that "tyrothricin affected both parasitized and unparasitized red cells as indicated by the fact that in stained films the red cells often stained more faintly than in untreated chickens and were folded or bent on themselves." They also observed that in all of the samples tested tyrothricin caused a partial hemolysis of the erythrocytes which was roughly proportional to its concentration. It was interesting to note that both tyrothricin and quinine inhibit the respiration of parasitized red cells as compared to untreated controls and that tyrothricin is more effective than quinine. It is suggested that these erythrocytic changes may have been associated with the antimalarial activity of this drug.

Atabrine

The chemotherapeutic action of atabrine was elaborated by Kikuth in 1932. The chemotherapeutic index of it and plasmochin was found to be about the same. However, in absolute figures, atabrine was four times as effective as quinine but fifteen times less effective than plasmochin (Temkin and Ramsey, 1944). Mackeras and Ercole (1949) state that atabrine appears to act by inhibiting the metabolism of hemoglobin. It has no action on parasites whose growth is independent of this protein nor on gameocytes which have already completed their growth. These investigators point out that atabrine is defenseless against exoerythrocytic forms of parasites and parasites that are subcutaneously inoculated until they appear in the circulating blood. This drug is capable of inhibiting the oxygen uptake of the malarial parasites (Coggeshall and Maier, 1941). It may also inhibit oxidative processes in the metabolism of the malaria parasites particularly those concerned with the oxidation of lactic and pyruvic acids (Speck and Evans, 1945).

That atabrine may be injurious to the hemopoietic system is shown by the observation of Custer (1946) who reported

fifty-seven fatal cases of aplastic anemia resulting from atabrine therapy. Three cases of Blackwater Fever have been observed to follow small doses of atabrine (Foy and Kondi, 1937–38). No observations have been made, however, to show that atabrine injured red cells directly, although the drug does enter erythrocytes (MaeGraith, 1946). The concentration of atabrine within the chicken's red cell is higher than it is in the plasma (Parmer, 1948).

Methylene Blue

A new chapter in the history of antimalarial drugs began in 1891 when Guttman and Ehrlich (1891) introduced methylene blue as an antimalarial agent. Methylene blue is also important since it was the starting point in the synthesis of plasmochin (Temkin and Ramsey, 1944). In considering the effect of antimalarial drugs on the erythrocytes, it may be significant to recall that methylene blue will produce methemoglobin and also that it is used in the treatment of methemoglobinemia. Methylene blue forms methemoglobin by catalytic oxidation. The dye reacts directly with hemoglobin to form methemoglobin and the reduced (leuco) form of methylene blue formed by this reaction reacts with oxygen to regenerate methylene blue. It is not equally clear, however, how methylene blue accomplishes reduction of methemoglobin to hemoglobin (Wendel, 1939). In an oxidation reduction system methylene blue replaces cytochrome (Brody, 1949). Methylene blue may produce an hemolytic anemia in dogs (Huyghebaert, 1924).

Ascorbic Acid

Ascorbic acid, of course, is not an antimalarial drug. However, this vitamin has been observed to play a role in *P. knowlesi* infection in the monkey. McKee and Geiman, in 1946, observed an abnormal course of the parasitemia in seven monkeys that showed spontaneous ascorbic acid deficiency and in two other animals which were made ascorbic acid deficient by removing this vitamin from their diet. Hemoglobinuria has been observed in humans who had a low plasma level of ascorbic acid. This symptom was relieved, however, following the administration of ascorbic acid (Fisher and Price, 1949).

Gibson and Harrison (1947) are of the opinion that the use of ascorbic acid in the treatment of methemoglobinemia appears to be limited to those cases in which the normal enzyme systems responsible for reducing methemoglobin are absent or suppressed. Based upon the observation that *P. knowlesi* infection is decreased in ascorbic acid deficient monkeys, one might conclude that the normal enzyme system within the red cell responsible for the reduction of methemoglobin is also necessary for the growth and development of this plasmodium. Cox and Wendel (1942) have expressed the opinion that the reduction of intracorpuscular methemoglobin is mainly, if not entirely, a function of the enzyme system contained within the erythrocytes. A high concentration of ascorbic acid within the erythrocyte serves as a substitute for the missing reducing system.

Discussion

The data compiled by Wiselogle (1946) on the war time research gives much information on those agents studied during that period. Other drugs have been studied since this was published. Boyd's (1949) recent and excellent publication summarizes our present knowledge relative to the most important antimalarial drugs. Apparently there still is much to be learned with regard to the mode of action of the majority of antimalarial agents. The ideal drug still has not been found (Boyd, 1949). The observation that many of the drugs used to treat malaria either in experimental animals or man will produce changes within the erythrocytes would suggest that maybe an important mode of action upon the parasite is one that is secondary to the effect of the drug upon the erythrocyte. As has been suggested by others, the effect of some drugs apparently is upon an enzyme system within the red cell, while other drugs may affect the consumption and transportation of oxygen by the erythrocytes which of itself could be detrimental to the parasite.

Methemoglobinemia has been observed following the giving of quinine, pamaquine, pentaquine, sulfanilamide and methylene blue. The severity and the frequency of occurrence of hemoglobinemia varies widely. However, it is significant that it has been observed to occur. Heinz-Ehrlich bodies have been

described in erythrocytes following the administration of pamaquine and sulfanilamide. The presence of such bodies in the red cell is also indicative of some change within the hemoglobin. Although atabrine may be injurious to the hemopoietic system, apparently no observations have been made to show this drug injures red cells directly. An hemolytic anemia has been observed following the use of quinine, pamaquine, pentaquine and tyrothricin.

The fact that many of the antimalarial drugs affect directly the red cell would lend support to the idea that their action is through the red cells and not by stimulation of immune reactions.

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EFFECT OF MERCURIAL DIURETICS UPON THE RESPIRATION OF THE RAT HEART AND KIDNEY

I. Effect of Mercuhydrin and Its Fractions

BELLE RUSKIN, WIKTOR W. NOWINSKI AND ARTHUR RUSKIN*

The direct effect of mercurial diuretics upon the isolated heart is that of producing conduction defects (Ruskin and Johnson, 1949). The diuretic action is, likewise, due to direct depression of renal tubular reabsorption (Handley et al., 1949) of sodium and water, among other possible mechanisms. That these pharmacological effects may be due to mercurial combinations with, and inactivations of, enzymes (Barron and Kalnitsky, 1947) involved in aerobic and anerobic metabolism, is supported by several sets of data. The cardiodepressive (conduction) and cardiolethal cardiac actions of mercurial diuretics are prevented and reversed by 2, 3 dimercaptopropanol (BAL) (Ruskin and Johnson, 1949). Handley (1949) demonstrated that non-lethal doses of meralluride sodium (mercuhydrin) depressed in vivo the succinic dehydrogenase of the kidney. In vitro, the drug inhibited the enzyme in both heart and kidney homogenates. Goth et al. (1950) presented evidence that various mercurial preparations repressed renal adenosine triphosphatase.

The present paper deals with effect of mercuhydrin upon the oxygen uptake of rat heart and kidney slices. The demonstrated depression presumably includes inhibition of various enzymes, including succinic dehydrogenase, involved in cellular oxidations.†

Method

The respiration was measured by the oxygen uptake, using the Warburg technique. The flasks contained Sörensen phos-

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phate buffer, at pH 7.2, with 0.2 to 0.3% glucose. To each experimental flask was added 10 mg of mercuhydrin (5.9×10^{-3} M final concentration), 10 mg of mercurated allylsuccinylurea (8.1×10^{-3} M), 9.5 mg of theophylline sodium (1.9×10^{-2} M), or 10 mg theophylline ethylene diamine (aminophyllin) (8.5×10^{-3} M), respectively.

Young adult rats, kept on standard diets, were used throughout. The animals were killed by a blow on the head, and the organs removed immediately and placed in chilled Ringer solution.

The heart slices were cut more than 0.5 mm. thick, and one slice only placed in each vessel (Potter, 1948). From the kidneys only the cortex was used.

The experiments were carried out at 38°C, the readings being taken every 5 minutes.

TABLE 1

Effect of mercuhydrin and its fractions on Qo_2 of rat heart slices

Controls (49)					
— 3.8	— 3.8	— 7.3	— 6.9	— 9.8	—15.3
— 4.2	— 5.8	— 8.2	— 4.5	— 9.0	—13.9
—10.0	— 4.1	— 4.6	—15.7	—12.2	—10.1
— 5.6	— 2.6	— 9.8	— 7.8	—11.3	—11.2
— 8.5	— 5.0	— 5.8	— 5.1	— 9.7	— 6.2
— 6.6	— 3.7	—12.1	— 3.5	— 7.5	—20.0
— 6.6	— 7.9	— 5.6	— 9.7	—18.1	— 9.4
— 2.7	— 6.8	—20.0	—13.2	—18.4	— 8.7
					— 6.3

Aver. — 9.0

Mercuhydrin (43)			Mercurated allylsuccinylurea (17)	Theophylline Sodium (16)	Aminophyllin (13)
— 3.4	— 4.0	— 2.0	— 4.1	— 2.9	
— 4.9	—12.9	— 3.5	— 3.0	— 5.6	
— 2.3	— 7.9	— 4.1	— 4.4	— 3.6	
— 2.4	— 3.9	— 3.2	— 2.5	— 4.6	—14.0
— 2.6	— 4.2	— 2.8	— 3.9	— 4.5	—10.8
— 2.4	— 5.1	— 3.9	— 6.7	— 6.6	— 9.8
—16.4	— 2.9	— 4.9	— 4.4	— 5.9	—14.8
— 2.2	— 2.5	—10.4	— 3.8	— 6.4	— 8.7
— 1.9	— 5.1	— 4.2	— 9.8	— 5.8	— 8.5
— 4.2	— 3.0	— 1.9	— 4.7	— 6.5	—12.0
— 2.0	— 6.5	— 8.3	— 5.5	— 5.3	—11.2
— 3.5	— 2.6	— 3.6	— 4.2	— 6.0	— 6.5
— 7.4	— 3.0	— 3.5	— 3.5	— 6.0	— 9.7
— 2.6	— 4.7	— 5.9	— 3.3	— 7.3	— 9.9
		—20.7	— 3.5	— 5.1	— 9.1
			— 3.7	— 5.6	— 7.7
			— 4.7		
Aver.	— 4.9		— 4.5	— 5.5	—10.2

Results

Heart. The results on the heart are summarized in Table I, from which it follows that the average Qo_2 for normal rat heart is — 9.0, a figure which corresponds closely to that of other authors (Pearson et al., 1949). The addition of mercuhydrin inhibits the oxygen uptake (Qo_2 — 4.9) by about 45%.

Because the mercuhydrin molecule is composed of mercurated allylsuccinylurea and theophylline ethylene diamine, it seemed important to establish whether the mercurial or the xanthine portion was responsible for the depression of the oxygen uptake. Investigated separately, the mercurated allylsuccinylurea gave an average Qo_2 — 4.5, very close to that of mercuhydrin (—4.9).

Theophylline sodium depressed the average Qo_2 of the heart slices (— 5.5) by about 39%. Aminophyllin (theophylline-ethylene diamine), on the other hand, gave approximately the normal value (Qo_2 — 10.2).

Kidney Cortex. In Table II are reported the results of the kidney experiments. The kidney slices alone gave an average

TABLE 2

Effect of mercurated allylsuccinylurea, theophyllin sodium and aminophyllin on Qo_2 of rat kidney cortex

Controls (16)	Mercurated allyl-succinylurea (12)	Theophyllin Sodium (24)		Aminophyllin (11)
—14.2				
—18.7				
—13.2				
—18.5				
—16.8	— 8.7	—10.3	—20.2	
—12.5	— 6.6	— 8.5	—21.0	—16.6
—19.7	—15.4	— 9.8	—17.4	—14.9
—19.5	— 8.1	—14.1	—17.0	—17.3
—25.3	— 7.3	—14.1	—25.1	—17.6
—29.6	— 8.8	—15.3	—17.2	—17.8
—16.8	—11.0	—14.2	—20.3	—18.2
—14.0	— 8.3	—18.0	—18.9	—15.0
—21.3	— 8.5	—18.5	—21.9	—21.3
—13.3	— 7.6	—10.3	—16.7	—14.4
—20.0	— 6.3	—21.4	—21.2	—16.6
—20.1	— 7.0	—26.3	—18.2	—21.0
Aver. —18.3	— 8.6		—17.3	—17.4

Qo_2 — 18.3, which is the normal value for kidney as found also by other authors (Holmgren and Naumann, 1947). In the case of added mercurated allylsuccinylurea, there was an inhibition of 53% (average Qo_2 — 8.6).

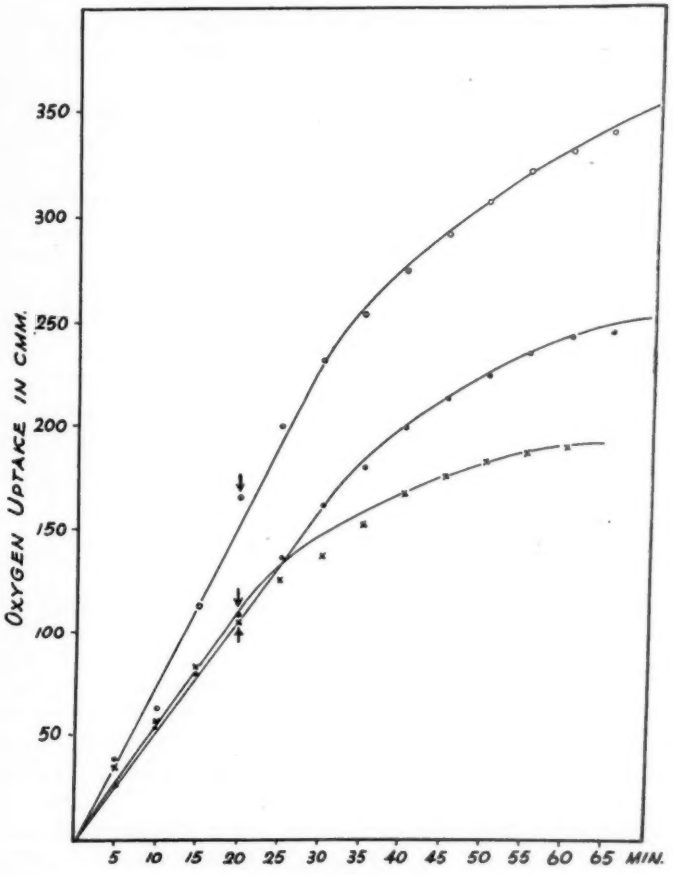

FIGURE 1

Influence of mercuhydrin, added during the experiment, upon the oxygen uptake of kidney slices. The tipping in of mercuhydrin is marked with arrows.

Estimations of oxygen uptake were also made by adding the mercurial compound, not immediately, but by tipping from the side arm during the experiment. The oxygen uptake of the kidney slices was measured during the first 20 minutes, and then the compound added. Three typical examples of this kind of experiment are shown in Fig. I, from which it follows clearly that the addition of the compound decreases the oxygen uptake (plotted in cu. mm. against time). Identical results were obtained in 14 experiments. The corresponding average figures were $Qo_2 - 22.8$ for the first 20 minutes and -11.1 after tipping. This 51% inhibition corresponds very closely to the 53% depression obtained in experiments in which the mercurial compound was added to the vessel from the start. From Fig. I it follows, furthermore, that there is a latent period of about 10 minutes before the depression of oxygen uptake becomes evident. It is difficult to interpret this fact. It is interesting to note, however, that a similar latent interval of 10 minutes was observed in the action of mercurials upon the isolated rabbit heart (Ruskin and Johnson, 1949).

Results with theophylline sodium gave a $Qo_2 - 17.3$, which is practically identical with the controls. Theophylline ethylene diamine, likewise, failed to depress the respiration of kidney slices ($Qa_2 - 17.4$).

Discussion

Our demonstration that mercuhydrin depresses cellular respiration in heart and kidneys is consonant with the data of other workers (Barron and Kalnitsky, 1947; Handley et al., 1949; Goth et al., 1950), indicating inactivation of various enzyme systems by mercurial compounds. The doses used to produce the depression of respiration were admittedly very large, and the inhibition failed to appear with doses 1/10 or less than those used.

The cardiac depression of respiration is primarily due to the mercurial fraction, since the other component of mercuhydrin, theophylline-ethylene-diamine, does not affect it. As some inhibition of oxygen uptake is seen following theophylline, we assume that the presence of ethylene diamine in the aminophyllin molecule neutralizes this effect.

In the kidney the results were more clear cut, indicating that the mercurial fraction, and not the theophylline or aminophyllin groups, inhibit the oxygen uptake. According to Goth et al. (1950), adenosine triphosphate is inhibited in the kidney by theophylline, as well as by mercurial compounds. The failure, however, of theophylline and aminophyllin to depress the cellular respriation inthe kidney may not contradict the results of Goth et al.

From the standpoint of the mechanism of mercurial diuresis, partial inhibition of cellular respiration of the kidney by mercuhydrin may favor the theory of decreased tubular reabsorption (Handley et al., 1949), since the oxidative enzymes play a role in tubular transport mechanisms. In itself, hypoxia, such as would seem to follow the depression of cellular respiration by mercurials generally tends to produce diuresis (Berger et al., 1949), and can, therefore, be invoked as an intermediate in mercurial diuresis. On the other hand, the failure of aminophyllin or theophyllin to deplete the oxygen uptake of the kidney may rather favor the theory of increased glomerular filtration than that of decreased tubular reabsorption to explain xanthine diuresis.

Summary

1. Mercuhydrin and the mercurial fraction of its molecule inhibit the respiration of rat heart slices 45 to 50%, and of kidney cortex slices 50%, as measured by the Warburg technique.
2. Theophylline inhibits the oxygen uptake of heart slices to a lesser extent than mercuhydrin, but does not influence the respiration of kidney slices.
3. Aminophyllin does not inhibit the respiration of either heart or kidney.
4. The conclusion is drawn that mercuhydrin inhibits the oxygen uptake of the heart and kidney primarily through its mercurial component.
5. The blocking of renal tubular oxidative processes by mercuhydrin in relation to its diuretic properties is discussed.

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EFFECT OF MERCURIAL DIURETICS UPON THE RESPIRATION OF RAT HEART AND KIDNEY SLICES

II. The Effect of Thiomerin

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In the previous paper of this series (Ruskin, Nowinski, and Ruskin, 1950) it was found that mercuhydrin depressed the respiration of rat heart and kidney slices, and that the effect was due primarily to the mercurial fraction. On the other hand, it is known that mercaptomerin sodium (thiomerin) requires doses containing 160 to 300 times more in mercury content than meralluride sodium (mercuhydrin) in order to achieve the same effects on cardiac conduction (Lehman 1947; Ruskin and Johnson, 1949). Although the cardiac and local (Ruskin, Rabinowitz, and Damiani, 1950) toxicity of thiomerin is comparatively low, its diuretic potency is just as great as that of mercuhydrin (Ruskin, Johnson, and Roddy, 1940; Grossman et al., 1950). Thiomerin, like other mercurial diuretics, apparently acts by decreasing the tubular reabsorption of water and salts, leaving the glomerular filtration and renal plasma flow unchanged (Grossman et al., 1950). It was therefore, of interest to compare the inhibitory effects of thiomerin with those of mercuhydrin and to evaluate its cardiac and renal effects from the standpoint of cellular respiration in the heart and kidney.†

Method

The details of the method are described in the previous paper (Ruskin, Nowinski, and Ruskin, 1950). 10 mg. of thiomerin was added to each flask, to make a 6.6×10^{-3} M final concentration.

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Results

The average Qo_2 of heart slices with thiomerin was — 6.0, a drop of 33% as compared with the controls (Qo_2 — 9.0); the average Qo_2 of kidney slices with thiomerin was — 8.9, a drop of 50% (Table I).

TABLE 1

Effect of thiomerin on Qo_2 of rat heart and kidney slices

Heart Slices		Kidney Slices	
Control (21)	Thiomerin (26)	Control (16)	Thiomerin (10)
	— 5.0		
	— 4.9		
	— 5.1		
	— 5.4		
	— 4.7		
— 5.1	— 4.4		
— 3.5	— 5.4		
— 9.7	— 4.1		
—13.2	— 4.9		
— 9.8	— 6.2		
— 9.0	— 4.8	—14.2	
—12.2	— 5.0	—18.7	
—11.3	— 5.2	—13.2	
— 9.7	— 6.7	—18.5	
— 7.5	—12.4	—16.8	
—18.1	— 5.8	—12.5	
—18.4	— 4.3	—19.7	— 5.7
—15.3	— 5.4	—19.5	— 8.5
—13.9	— 9.0	—25.3	— 6.3
—10.1	— 4.8	—29.6	— 9.2
—11.2	—11.8	—16.8	— 9.6
— 6.2	— 8.0	—14.0	— 8.7
—20.0	— 7.3	—21.3	— 8.4
— 9.4	— 6.0	—13.3	— 9.6
— 8.7	— 5.2	—20.0	— 9.8
— 6.3	— 5.0	—20.1	—13.1
Aver. — 9.0	— 6.0	—18.3	— 8.9

Discussion

Thiomerin depressed the respiration of rat heart slices about 33% as compared with 45% for equivalent (in mercury content) concentrations of mercuhydrin (Ruskin, Nowinski, and Ruskin, 1950). This corresponds to the lesser cardiotoxicity of thiomerin (Lehman, 1947), but not to the actual cardiolethal (1:1,000) and cardiotoxic (1:300) ratios in isolated rabbit hearts (Ruskin and Johnson, 1949). Possibly other biochemical

reactions are responsible for the difference in cardiac tolerance of the two drugs. The presence of the monothiol component in thiomerin, contrasted with the absence of SH groups in mercuhydrin, renders thiomerin less likely to combine with, and inactivate, sulfhydryl-containing oxidative enzymes in the heart and kidney, and, in consequence, presumably less toxic to both. Actually, the depression of respiration of the kidney cortex following thiomerin was the same ($Qo_2 — 8.9$) as following mercuhydrin ($Qo_2 — 8.6$) (Ruskin, Nowinski, and Ruskin, 1950). Others (Handley et al., 1959; Orth et al., 1950) have reported greater depression of glomerular filtration from repeated doses of thiomerin than mercuhydrin in animal experiments. From the standpoint of cellular oxidations, however, as from clinical studies (Ruskin, Johnson, and Roddy, 1949), the conclusion can be drawn that thiomerin and mercuhydrin have equivalent nephroinhibitory and nephrotoxic effects. This does not exclude different effects of the two drugs upon the anaerobic metabolism of the kidney, which may be of more importance in such conditions as circulatory failure, and which is under study.

Summary and Conclusions

1. Thiomerin inhibits the respiration of rat heart slices about 1/3 less than mercuhydrin, in doses with equivalent mercury content.
2. The inhibition of renal respiration is the same for both drugs.
3. These observations support, in part, the lesser cardiotoxicity of thiomerin, and the clinical equivalence in diuretic and nephrotoxic effects of the two mercurial compounds.

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PATHOLOGICAL DIAGNOSES OF UTERUS AND OVARIES IN 6042 CASES OF PELVIC SURGERY AT SCOTT AND WHITE CLINIC*

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In the course of obtaining material for a morphological study of malignant ovarian neoplasms, 6042 cases in which either the uterus, one or both ovaries, or the uterus and ovaries examined by a pathologist were studied. This investigation covered the cases operated at Scott and White Clinic between 1925 and the first quarter of 1949. The majority of the patients came from the southwestern United States: namely, Texas, New mexico, and Oklahoma. Practically all of the cases were individuals of the Caucasian race. The age was not correlated. No clinical data is presented in this series, since this is only a listing of the pathological diagnoses. Diseases of the cervix uteri and the fallopian tubes are not included.

TABLE 1

Benign Neoplasms of the Ovaries

	Total Cases	Percentage Benign Ovarian Neoplasms (377 Cases)	Percentage Ovarian Tumors Benign and Malignant (541 Cases)	Percentage Total Ovaries (3474 Cases)	Percentage Total Cases (6042 Cases)
Cystic	324	86	60.0	9.3	5.4
Pseudomucinous	23	6.1	4.3	0.7	9.4
Serous	9	2.3	1.7	0.3	0.1
Unclassified	144	38.2	26.7	4.1	2.4
Dermoid	148	39.3	27.4	4.3	2.4
Solid	53	14.0	9.8	1.5	0.9
Fibroma	50	13.3	9.2	1.4	0.8
Brenner	2	0.5	0.4	0.06	0.03
Angioma	1	0.3	0.2	0.03	0.02
	377	100.0	69.7	10.9	6.2

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The ovaries were examined in 3474 cases, and the uterus in 4653. This includes 2085 cases in which both the uterus and one or both ovaries were removed. The distribution of the various distases encountered is presented in tabular form. Table I lists the benign neoplasms of the ovary. The tumors of the ovary are classified according to Novak (1947).

The malignant ovarian neoplasms are listed in Table II.

TABLE 2

Primary Malignant Neoplasms of the Ovaries

	Total Cases	Percentage Malignant Ovarian Tumors (164 Cases)	Percentage Ovarian Neoplasms (541 Cases)	Percentage Total Cases Ovaries Examined (3474 Cases)	Percentage Total Cases (6042 Cases)
Carcinomas	157	95.7	29.0	4.5	2.6
Solid Carcinoma	61	37.2	11.3	1.8	1.0
Adenocarcinoma	36	22.0	6.7	1.0	0.6
Papilarry	10				
Non-Papillary	26				
Alveolar	6	3.7	1.1	0.2	0.1
Scirrhous	1	0.6	0.2	0.03	0.02
Plexiform	1	0.6	0.2	0.03	0.02
Medullary	1	0.6	0.2	0.03	0.02
Granulosa Cell	9	5.5	1.7	0.30	0.1
Thecoma	5	3.0	0.9	0.10	0.08
Leutoma	2	1.2	0.4	0.05	0.03
Cystic Carcinoma	96	58.5	17.7	2.8	1.6
Pseudomucinous	16	9.8	3.0	0.5	0.3
Serous	80	48.8	14.7	2.3	1.3
Sarcoma	6	3.7	1.1	0.2	0.1
Teratoma	1	0.6	0.2	0.03	0.02
	164	100.0	30.3	4.7	2.7

The occurrence of 164 (30.3%) primary malignant neoplasms of the ovary in a series of 541 tumors, both malignant and benign, compares with the incidence of 110 (36.4%) malignancies in 302 cases of ovarian neoplasia cited by Lynch (1936).

Tables III and IV list the non-neoplastic conditions of the ovary. Table V depicts the metastatic malignancies of the ovaries.

TABLE 3

Non-Neoplastic Cysts of the Ovary

	Total Cases	Percentage (2175 Cases)	Percentage Total Ovaries (3474 Cases)	Percentage Total Cases (6042 Cases)
Follicle	334	15.4	9.6	5.5
Corpus Luteum	119	5.5	3.4	2.0
Hemorrhagic	189	8.7	5.4	3.1
Endometrial	102	4.7	2.9	1.7
Germinal Inclusion	7	0.3	0.2	0.1
Unclassified	129	5.9	3.7	2.1
Simple Cyst*	1,295	59.5	37.2	21.4
	2,175	100.0	62.6	36.0

*Simple Cysts and functional ovaries removed incidental to pelvic surgery.

TABLE 4

Miscellaneous Conditions of the Ovary

	Total Cases	Percentage (750 Cases)	Percentage Total Ovaries (3474 Cases)	Percentage Total Cases (6042 Cases)
Atrophy	304	40.5	8.8	5.0
Inflammation	384	51.2	11.1	6.4
Fibrosis	44	5.9	1.3	0.7
Normal	13	1.7	0.4	0.02
Calcified	5	0.7	0.1	0.008
	750	100.0	21.6	12.4

TABLE 5

Metastatic Malignant Tumors of the Ovary

	Total Cases	Percentage Neoplasms Ovary, Primary and Metastatic (549 Cases)	Percentage Total Ovaries (3474 Cases)	Percentage Malignant Primary and Metastatic (549 Cases)	Percentage Total Cases (6042 Cases)
Krukenberg	2	0.4	0.06	1.2	0.03
Unclassified carcinoma	3	0.6	0.09	1.8	0.05
Sarcoma	3	0.6	0.09	1.8	0.05
	8	1.5	9.2	4.8	0.13

In Table IV are listed the diseases of the uterus in the order of frequency of occurrence. There were a total of 160 malignant tumors of the uterus. It is again emphasized that this study does not include the cervix. The incidence of malignancy of the uterus was 3.4% of all uteri examined. Adenocarcinomas were most common, 135 cases as compared with sarcoma, 23 cases, a ratio of over 5:1. Malignancies of the uterine fundus constituted 2.6% of the 6042 cases. Malignancies of the ovary made up 2.7% of the cases, thus 5.36% of the 6042 cases had a malignant process either of the ovary or the uterus. The 160 malignant neoplasms of the uterus constituted 3.4% of all the uteri. Leiomyomata constituted the majority of the benign tumors, 2564 cases or 55.1% of all uteri and 42.4% of all the cases in this series. There were 288 cases (6.2% of uteri) of adenomyosis.

TABLE 6

Conditions of the Uterus

	Total Cases	Percentage Uteri (4563 Cases)	Percentage Total Cases (6042 Cases)
Leiomyoma	2,564	55.1	42.4
Miscellaneous (Fibrosis, Hypertrophy, Myometritis, etc.)	746	16.0	12.3
Normal	485	10.4	8.0
Adenomyosis	288	6.2	4.8
Atrophy	267	5.7	4.4
Adenocarcinoma	135	2.9	2.2
Endometrial "Polyps"	43	0.9	0.7
Hyatid Mole	26	0.6	0.4
Pregnant	25	0.5	0.4
Sarcoma	23	0.5	0.4
Irradiation Reaction	17	0.4	0.3
Bicornute	16	0.3	0.3
Infantile	14	0.3	0.2
Chorioepithelioma	2	0.05	0.03
Tuberculosis	2	0.05	0.03
	4,653	100	77.0

SUMMARY

The pathologic diagnoses made on uteri and ovaries removed surgically in 6042 cases over a 24-year period are presented in tabular form.

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THE EFFECT OF ASCITIC FLUID AND OF BLOOD SERUM ON THE OUTGROWTH OF EPIDERMAL CELLS FROM THE HUMAN SKIN IN TISSUE CULTURE*

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Utilizing phase-contrast microscopy, the morphological features of living epidermal cells grown in tissue culture from explants of human split-skin grafts have been described in a preliminary paper by Lewis, Pomerat, and Ezell (1949). The present study was designed to compare the growth-promoting properties of Gey's solution, autologous and homologous blood serum, cord serum and human ascitic fluid from patients with malignant *versus* non-malignant processes when used as the fluid nutrient in hanging-drop cultures of human adult skin. For the purpose of this study, culture conditions have been maintained as constant as possible while variations in incubation temperature, types of glassware, bacteriostatic agents and other factors have not been explored.

Materials and Methods

Skin for tissue cultures was obtained from young adults in a good nutritional state on whom grafting operations were being performed. Routine surgical procedure included skin preparation by washing with green soap and water for two minutes, painting with 0.5% iodine in 70% alcohol, removing all visible traces of iodine with 70% alcohol and washing with ether; and "free hand" sectioning or removal of skin with the Padgett-Hood dermatome. We have found the "free hand" technique preferable since it usually makes possible thinner strips of epidermis. Epithelial outgrowth *in vitro* appears to be greatest when the amount of dermis does not greatly exceed that of the epidermis in the explanted tissue. Surgical specimens

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were kept moist with saline in a gauze sponge in a sterile petri dish and set up in cultures within two hours after removal from the patient.

The problem of evaluating the amount of epithelial outgrowth from explanted tissues offers many difficulties. In a recent report from this laboratory (Blocker, Pomerat, and Lewis, 1950), it was suggested that the time for epithelial closure of a standard sized hole punched out of a square piece of skin might be utilized for quantitative studies. This can be done by using a 16 gauge needle which has been truncated and beveled on the luminal side to produce a sharp edge. Unfortunately, within 24 hours of incubation, such explants are often markedly distorted due to the contraction of variable amounts of elastic and collagenous fibers.

For the present study, pieces of skin approximately 1½ mm. square or circular "punch" explants were utilized. The latter were prepared with a 13 gauge needle which has been truncated and beveled on its outer surface. Using a slightly rotatory motion, circular disks of skin approximately 1.8 mm. in diameter were punched out of sheets of skin spread out on the surface of a thin strip of sterilized oak wood. While such explants developed some degree of distortion after explantation, greater uniformity of size could be obtained with this method than with the square pieces.

Cultures were made by placing one drop of chick embryo extract in the center of a No. 1 ⅞ in. square cover glass, transferring a skin explant, adding one drop of heparinized cockerel plasma containing 1,000 units of penicillin per ml. and stirring the mixture thoroughly. When the clot was firmly set, four drops of the various fluids to be tested were added. This quantity was found most suitable with the use of deep well slides (19 mm. in diameter and 2 mm. deep). All nutrients were delivered from 27 gauge needles affixed to 1 ml. tuberculin syringes.

Epithelial outgrowth, often proceeding in the form of long tongues, irregularly lobose sheets or migrations from only limited areas of the periphery of the explant, has been evaluated by counting the total number of cells when these are not too numerous or by assigning arbitrary values for varying degrees

of cellular extension (Parshley and Simms, 1950). In a preliminary series of experiments we utilized an arbitrary system of rating the amount of outgrowth. The margin of the explant was placed at the edge of the optical field in a microscope system giving a magnification of 100 x. Outgrowth extending entirely across the field was assigned the value of 25. Outgrowth extending across three-fourths of the field was rated at 20; one-half the field at fifteen; one-fourth at ten. A rating of five was given for a total of from 25 to 50 cells. No outgrowth was rated at zero. A total "score" could thus be determined for various series of experiments. While more than 3,000 cultures were studied with this technique in screening experiments it was realized that irregularities in the margin of the outgrowth required a more accurate system for obtaining quantitative results. The method of making systematic projections of growing cultures requires a considerable investment of effort but it was felt desirable to perform a few check experiments with this technique.

For experiments to be evaluated by the projection technique, 20 hanging-drop cultures were prepared for each condition. A 24-hour period of incubation, during which epithelial outgrowth did not take place, was allowed for changes in the form of the explant in its equilibration to the conditions of the medium. Each explant was then projected and outlined in pencil on a standard piece of 24 lb. Hammermill bond paper with the use of a "Rayoscope" set for a magnification of exactly 50x. In order to reduce the labor involved, one-half of the series was measured on "odd" days and the other half on "even" days until five recordings had been made for every culture. The paper-outlined tracings were cut out with scissors and the weight for the 20 cultures in each series was determined for each day of observation. The per cent increase in the area from the initial (24 hour equilibrated explant) was then calculated, using the formula:

$$\frac{o \times 100}{e} = \% \text{ of outgrowth}$$

with e the area of the explant after 24 hours of incubation and o the area of the explant (outgrowth) on successive days.

Figure 1. Outgrowth of epidermal cells in relation to various types of fluid media as measured by an arbitrary method of rating (see text).

DESCRIPTION OF SKIN DONOR		Gey's solution	Rabbit serum	Fowl serum	Autoserum	Homoserum	Cord serum	Non-malignant (Portal cirrhosis) ascitic fluid GO.	Non-malignant (Portal cirrhosis) ascitic fluid GR.	Non-malignant (Portal rhosis) ascitic fluid TU.	Malignant (carcinoma of peritoneal organs) ascitic fluid PA.	Malignant (scirrhous adenocarcinoma of stomach) ascitic fluid MO.	Malignant (Papillary adenocarcinoma ovary) ascitic fluid SM.
White, Female, 9 years old	RBC — 3,830,000												
Poor nutritional balance	Hb — 11.2 gm.												
Diag.: Kaloid formation after burn	WBC — 6,600			5		95		75					
Skin from thigh													
Negro, Male, 50 years old	RBC — 3,200,000												
Fair nutritional balance	Hb — 9.75 gm.												
Diag.: Skin and tendons injury on back of left hand	WBC — 9,200		10	35	52	125					162	170	
Skin from thigh													
White, Female, 36 years old	RBC — 4,400,000												
Good nutritional balance	Hb — 12.9 gm.												
Diag.: Burn deformity on left hand	WBC — 8,300	0			44	80	95	50	55	60	115	135	
Skin from abdomen													
White, Male, 32 years old	RBC — 5,360,000												
Good nutritional balance	Hb — 16.8 gm.												
Diag.: Burn deformity on dorsum, right hand	WBC — 6,900								32		82	105	
Skin from abdomen													
White, Male, 70 years old	RBC — 4,320,000												
Fair nutritional balance	Hb — 13.3 gm.												
Diag.: Carcinoma on left ear	WBC — 8,550				75	90	125	64			100	130	
Skin from abdomen													
White, Female, 16 years old	RBC — 4,600,000												
Good nutritional balance	Hb — 14.43 gm.												
Diag.: Burn scar on right hand	WBC — 11,300				50	90		40					188
Skin from right thigh													

Results

From a variety of preliminary experiments which were performed with the simple method of arbitrary numerical rating, Figure 1 represents only those results obtained on skin from six individuals. The tissues utilized were derived from split grafts taken from the abdomen or thigh and at least three different fluid nutrients were employed in the culture medium for each series of experiments.

It has been regularly observed that there is a great deal of variation in the amount of outgrowth from skin derived from different patients even though culture procedures and nutrient media are identical for all tests. This individual difference in skin behavior *in vitro* is a fundamental problem requiring further investigation.

Comparison of the outgrowth obtained from each individual in relation to the fluid nutrient which was employed leads to the following conclusions: no outgrowth occurred with the use of Gey's solution. In ascending order of activity, the fluid nutrients can be listed as rabbit serum, fowl serum, non-malignant, autoserum, homoserum, human umbilical cord serum and malignant ascitic fluid. Three different ascitic fluids of each of the non-malignant and malignant series were included. The physico-chemical features of these nutrients are presented in Figure 2. The methods utilized have been described in the report by Pomerat, Nowinski, and Rose (1950). No specific correlation has been established thus far between the growth-stimulating propensity of malignant ascitic fluid and any single or any group of chemical constituents.

Check experiments utilizing projection technique for evaluating outgrowth were conducted on skin from three different patients described in Figure 3.

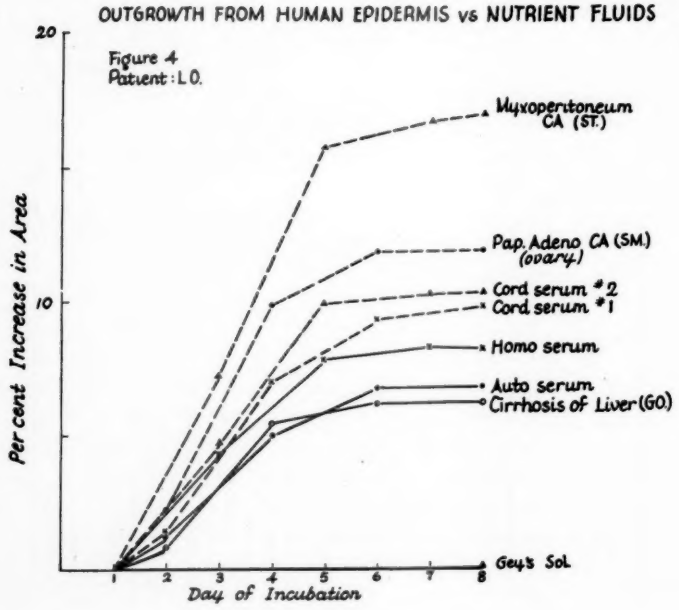
Again it will be seen that tissues from these different subjects showed varying growth potentialities in spite of the fact that in some instances the same nutrient fluid was utilized for the three tissues. Adequate explanation for the production of approximately three times greater cell migration from the tissue of patient MT. as compared with patients KI. and LO. has not yet been found.

Figure 2. Physicochemical features of ascitic fluids utilized as fluid nutrients for the cultivation of human skin.

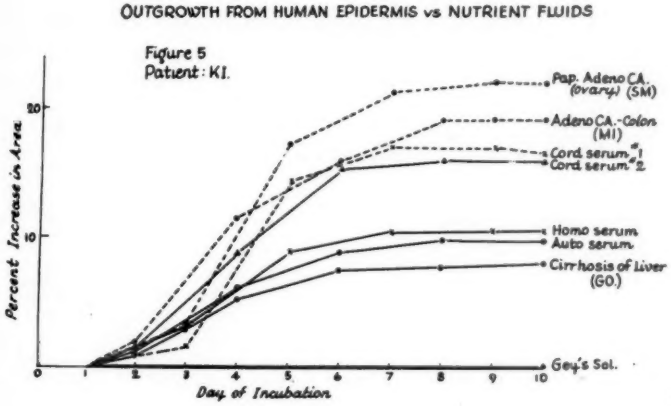
		GO. Non-malignant (Portal cirrhosis)	GR. Non-malignant (Portal cirrhosis)	TU. Non-malignant (Portal cirrhosis)	MO. Malignant (Scirrhous adenocarcinoma of stomach	SM. Malignant (Papillary adenocarcinoma of ovary)	PA. Malignant (Carcinoma of peritoneal organs)	MI. Malignant (Adenocarcinoma of colon)	ST. Malignant (Myxoperitoneum carcinoma)
Specific Gravity		1.006	1.010	1.010	1.022	1.022	1.012	1.013	1.017
pH		9.09	8.87	8.87	9.22	8.91	8.43	7.78	7.22
Non-protein Tyrosine	mg.%	8.99	13.02	15.55	16.07	11.78	14.26	11.45	17.50
Protein Tyrosine	mg.%	87.96	72.43	88.20	131.4	250.94	217.31	118.8	223.2
Inorganic Phosphorus	mg.%	10.58	8.22	7.35	11.68	8.23	traces	10.68	9.35
Pyro-Phosphorus	mg.%	11.17	16.47	16.70	26.02	21.75	19.40	67.52	7.99
Non-protein Nitrogen	mg.%	67.4	11.47	12.96	31.5	11.25	26.25	22.95	39.15
Protein Nitrogen	mg.%	339.5	359.0	280.95	74.7	712.00	278.00	147.68	542.1
Glucose	mg.%	20.30	18.61	18.46	8.65	19.74	24.25	16.73	20.77
Cholesterol		0	0	0	0	0	0	0	0
Gmelin		+	+	—	+	+	+	+	+
Alkaline Phosphatase	B.u.	0	116	116	73	0	4.6	115	73
Acid Phosphate	N.u.	2.5	6.66	4.17	6.7	13.34	0.17	5	7.5
Proteolysis		0	0	0	0	0	0	0	77
Sodium	MEg.			143.6					143.6
Potassium	MEg.			3.9					4.8
Calcium	mg.%	6.9							7.0

Figure 3. Description of skin donors for experiments in which outgrowth of epithelial cells in relation to various types of fluid media was measured by projection technique.

LO.	
White, Male, 45 years old	RBC — 4,850,000
Fair nutritional balance	Hb — 14.8 gm.
Diag.: Lymphedema right leg	WBC — 9,700
Skin from right leg	
KI.	
White, Male, 25 years old	RBC — 4,630,000
Fair nutritional balance	Hb — 14.4 gm.
Diag.: Gunshot wound, left hand	WBC — 7,000
Skin from abdomen	
MT.	
White, Male, 22 years old	RBC — 6,030,000
Good nutritional balance	Hb — 16.6 gm.
Diag.: Burn deformities on both hands	WBC — 8,650
Skin from abdomen	



Results shown in Figures 4, 5, and 6 confirm the conclusions obtained in experiments using the arbitrary system of rating outgrowth; namely, that ascitic fluid from patients with malignancies served as the most valuable nutrient factor of the series. Cord serum proved almost equally effective. Auto- and homosera were approximately half as growth-promoting while non-malignant ascitic fluid proved somewhat less useful than adult blood serum. It is important to note that while the three different charts showed different absolute values the various curves showed the same order of arrangement.

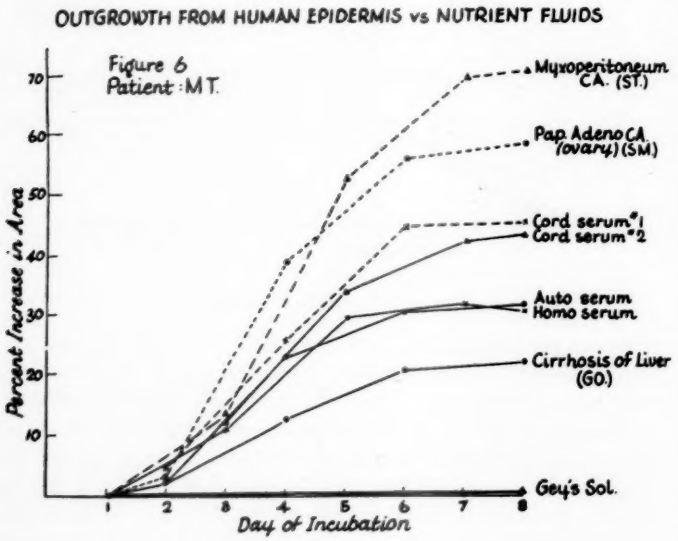


Summary and Conclusions

Ascitic fluid associated with malignant processes and human umbilical cord serum appears to exert a growth-promoting capacity approximately twice greater than either autologous or homologous blood serum when used in the fluid phase of culture medium in which adult human skin is grown in hanging-drop preparations. It has not yet been possible to identify physico-chemical characteristics in ascitic fluid which might be held responsible for its nutritional or growth-stimulating properties.

Provisional studies on the pH of hanging-drop cultures tested at varying times during the process of incubation do not suggest that extensive outgrowth is determined by this factor.

The role of electrolytes, pH and of amino-acids in relation to the growth of skin *in vitro*, will require further study under the conditions of our experimental methods. Parshley and Simms (1950) have suggested that optimum growth of epidermis occurred at a pH of 6.9 with the aid of a medium poor in calcium, rich in phosphate and in neutralized aspartic acid. These desiderata have been incorporated in their solution number Z16. Two parts of this solution are employed with one part of plasma or serum or serum ultrafiltrate and rectified with 4% CO_2 to a pH 6.9 for use as a fluid medium in Carrel flasks. Experiments in our laboratory are designed to compare this suggestion with our present technique of utilizing malignant ascitic fluid or cord serum as an ideal fluid phase for the promotion of epithelial outgrowth from explants of human adult skin.



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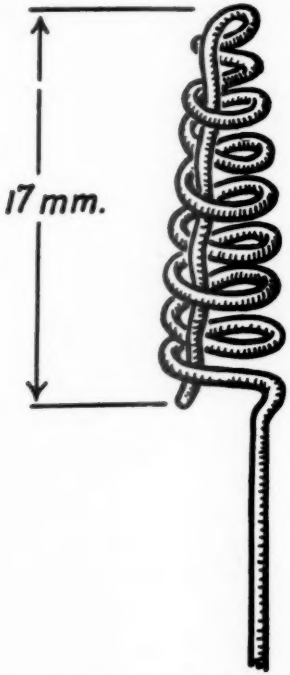
AN INOCULATING DEVICE FOR USE IN PROTOZOAN CULTURE

W. J. WINGO*

An inoculating device, which may be sterlized by flaming but which will transfer greater volumes of fluid than the ordinary bacteriologist's loop, is desirable for transferring protozoa grown under sterile culture conditions. The need for such a device recently became apparent in this laboratory, and a simple apparatus with the desired characteristics was made from 22 B & S gage nichrome wire. Figure 1 shows the finished inoculating spiral.



*From the Department of Biochemistry, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston, Texas. Submitted for publication June 5, 1950.

The point of an ice pick is a good mandrel for winding the spiral. A small loop is formed near the point of the pick, and about 1.5 inches of wire extends past the loop. This short length of wire is bent back against the shaft of the pick, and successive loops of the other longer end of the wire are then formed around the shaft in the direction of its increasing diameter. The turns of this spiral alternately pass over and under the short end of the wire, and the spiral is formed under considerable tension so that good contact between the spiral proper and the short end is obtained. Finally, the spiral is slipped off the ice pick, and the short end is cut off near the last turn of the spiral. The long handle of the spiral is held in a needle holder, and the device is ready for use.

To test the capacity of the spiral, the water picked up by it after being flamed and dipped in water was shaken off into a tared bottle and weighed. In four trials, 85, 89, 79, and 96 mg. of water were transferred. A 5 mm. loop made of the same kind of wire, in two trials, transferred 8.9 and 9.3 mg. of water. It is thus apparent that the spiral will permit the use of much larger inocular than will the loop.

No attempt was made to prepare a spiral having maximum capacity, but if even larger inocular are desired, it is obvious that several short spirals could be mounted side by side, and the volume of inoculum thereby increased.

INTEGRATION OF HOSPITAL SERVICES AND COMMUNITY PLANNING UNDER PUBLIC LAW 725

DEAN F. WINN*

Two points stand out in the philosophy behind Public Law 725; namely, (1) to supply good hospitals where they are most needed, and (2) to enable these hospitals to furnish adequate patient care. In accomplishing these objectives the State Agency is committed to the following interrelated activities:

a. Survey of existing hospitals to determine the need for additional facilities and to outline a co-ordinated hospital system.
b. Planning and construction of needed hospitals.
c. Standardization of these hospitals to insure good patient care.
d. Integration of hospital services in a co-ordinated hospital system.

Survey and State Plan

As a result of the original State-wide survey and subsequent special surveys a State Plan has been developed to carry out this mission. This plan establishes on paper a pattern for regionalization of the State. Each region is comprised of two or more general hospital service areas which can be closely related to provide better hospital care through co-operative effort. These hospital service areas currently contain, or will contain, one or more hospitals. The boundaries of the areas are drawn, in theory, to include the population which tends in the main to seek service from the hospital or hospitals located in the area. In Texas, county boundaries, or a combination of two or more counties, have been used to delineate service areas. The State Plan provides for a determination of the relative need of service areas for new hospitals or for expansion of existing installations.

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*From the Hospital Survey and Construction Division, Texas State Department of Health.

The States are required to distinguish three types of general hospital service areas—base, intermediate and rural—in accordance with the role each area would play in a regional co-ordinated hospital system.

Base Areas. A base area must have the following characteristics: (1) Irrespective of the population of the area, it must contain a teaching hospital of a medical school which must be suitable for use as a base hospital in a co-ordinated hospital system: or (2) the area must have a total population of at least 100,000 and contain, currently or on completion of the hospital construction program, at least one general hospital with at least 200 beds. This hospital must furnish internships and residencies in two or more specialties and must be suitable for use as a base hospital in a co-ordinated hospital system within the State.

Intermediate Areas. Such areas must have a population of at least 25,000 and contain, currently, or on completion of the program, at least one general hospital of 100 or more beds and which would be suitable for use as a district hospital in a co-ordinated hospital system.

Rural Areas. This term designates other general hospital service areas.

Planning

The next step has been the planning and construction of new hospitals or the expansion of existing facilities in these hospital service areas. Assistance in community planning of hospitals is a serious obligation of the State Agency if the construction of monuments to poor planning is to be avoided. At times it is necessary to discourage applicants when it appears that sparse population, questionable economic stability, personnel limitations, proximity to existing hospitals, lack of community spirit, and the like, would not justify the project. Justification based on civic pride and ill-conceived enthusiasm of Chambers of Commerce must be properly evaluated. The urge to provide a community hospital usually stems from a desire of the people to be hospitalized near their homes under the care of their own physicians. Not infrequently in small communities the people are convinced that unless hospital facilities are made available they will never succeed in securing or retaining competent doc-

tors in the community. Certainly it is true that if an adequate level of medical care is to be provided in an area qualified physicians must be willing to practice there. They are not willing to do so unless their legitimate desire for hospital affiliation can be satisfied. Perhaps this partly explains the large number of small private hospitals which have been constructed.

During the planning stage an effort is made to impress upon sponsors the obligations of the hospital to the community and the influence it will have in matters of public health. They are reminded that an application for hospital construction could not be approved unless the water supply of the city or town has been certified as meeting the standards of the State Health Department. It is not at all unusual that this requirement has forced city officials to undertake long-needed remedial measures. Thus the hospital sponsors are impressed with the knowledge that their hospital has begun to be a good influence in community health even before it is built. Methods of operation and maintenance and staffing problems are discussed. The significance of their agreement to operate the hospital when completed in accordance with minimum standards established by the State Agency is carefully explained. The part the hospital is intended to play, and the benefits it will receive, by participating in a co-ordinated hospital system is stressed.

In the case of county hospitals, either through necessity or inclination, the County Judge usually assumes the burdens of the construction stage. He may have the counsel of the busy local doctors or of a loosely organized hospital committee. The appointment of a Board of Managers is too often unduly delayed. As a rule the members of the Hospital Board are capable, public-minded citizens but totally unaware of their duties and responsibilities and without any knowledge of acceptable hospital organization or practices. The doctors and the lay sponsors alike are prone to think only in terms of the greatest number of beds for the money available and to ignore the importance of adequate space requirements for administration, service departments, storage, etc. It is necessary to stress repeatedly the importance of planning the hospital around the functions to be performed. This is particularly true concerning the purchase of equipment.

At these early conferences valuable contacts are made and confidence in the competence and helpful service of the State Agency is established—a matter of great usefulness in later conferences concerning hospital standarization.

Construction

Hospitals constructed under this Act must conform to minimum standards of construction, including safety factors and good functional arrangements of facilities. They must be also adequately equipped. All plans must be approved by the State Agency and by the U. S. Public Health Service prior to beginning construction.

To date, in Texas 23 hospitals totalling 674 new beds have been completed: :18 of these were constructed in rural areas; 22 additional projects totaling 1,288 new beds are under construction; 27 hospitals totalling about 3,800 new beds are in various stages of planning.

Standardization

I do not think that integration of hospital services alone can be expected to accomplish our purpose of providing better patient care. I do not believe that integration of hospital services can be successfully accomplished unless some way can be found to create a desire and a determination on the part of hospital authorities to meet recognized standards of operation and maintenance. Although the two approaches are intimately related, I believe that a definite program, aimed primarily at hospital standardization, should be established. Unless a hospital is willing to adopt minimum acceptable standards of organization and operation, it is unlikely that it will maintain a sustained and effective interest in integrated hospital services. The efforts of national and State organizations directed at standardization of hospitals have accomplished much but not enough, particularly in the smaller hospitals.

The completion of twenty-three hospitals and the active construction of an additional twenty-two has brought into sharp focus the tremendous challenge confronting the State Agency of assisting these hospital authorities in fulfilling their obligation to adopt basic acceptable standards. To this end the Texas

State Agency has engaged in an intensive educational campaign conducted largely by personal interviews with hospital boards, medical staffs and administrators. At these conferences basic acceptable standards are preached and explained in detail. Suggestive literature, including model by-laws, rules and regulations, is put into the hands of individual members who appear to possess an outstanding interest and qualities of leadership. The State Agency is indebted to Dr. MacEachern, to the American Hospital Association and to the Duke Foundation for supplying much of this material. The desirability of divorcing the hospital from local politics is discussed. Qualification for recognition by national organizations is stressed. Emphasis is placed upon the importance of the rural hospital establishing liaison with larger hospitals for the type of assistance as visualized in an integrated service program. The cordial reception given the representatives of the State Agency at these conferences and the desire of governing boards and administrators to learn and to co-operate has been most gratifying and stimulating.

This approach to bringing about good hospital organization and adequate patient care is considered absolutely essential because of the lack of fundamental information on the part of hospital board members and because of the indifference and complacency so frequently encountered among physicians in rural communities regarding organized medical staffs, adequate medical records, and the like. It is a futile gesture to depend upon the mailing out of pertinent information. The suggestions will not be read, or will not be understood, and little action will result. The "whys" and the "hows" of good organization must be painstakingly explained and opposition from local physicians broken down.

Personnel

Fair success has been experienced in the matter of securing administrators, and arrangements have been made for their further training in Institutes sponsored by the State Agency.

In some areas personnel limitations, especially regarding qualified doctors, to form an adequate medical staff, are a

serious problem. The State Medical Association has been helpful in locating doctors in rural areas.

It is surprising how many hospitals have been able to find trained nursing personnel locally to supplement the nursing staff. Active formal training programs for volunteer nursing aides are being conducted in some of the hospitals.

Much difficulty has been encountered in securing qualified laboratory technicians. Arrangements have been made for hospitals to send their technicians to the State Health Department laboratory for supplementary training. Many of the hospitals are sending their tissue specimens to central laboratories. Some have laboratory and X-ray consultants from large hospitals engaged to make periodic visits to check methods and results. In connection with the general problem of clinical laboratories, it is interesting to note that of 558 laboratories in the State to which test serology specimens were submitted by the State Board of Health under the recently enacted premarital and pre-natal law, only 33 1/3% qualified for certification.

A comprehensive check list, or record of achievement, similar in content to the Scoring Report of the American College of Surgeons, but without point rating, is maintained on each hospital. This has been supplemented by a manual entitled "Elements of Hospital Operation" which has been published recently by the U. S. Public Health Service. These records of achievement indicate that the results being obtained by this educational campaign fully justify the effort and the method. They will furnish eventually useful source information on the problems of opening new hospitals and experience data not now available. Good organization is being effected and many of the hospitals have arranged for consultant aid with regional base hospitals. Staff relationship is being developed along acceptable lines and administrators are putting their house in order. The State Agency is preparing to inaugurate regional or State-wide Institutes for the benefit of hospitals in the construction program. The Institutes will be conducted by experienced leaders in the field and opportunity will be given for free discussion.

Integration

So much for planning, construction and standardization. This brings us to the difficult question of putting life into the ideal of integration of hospital services. It has already been shown how, under Public Law 725, a pattern on paper has been set up for a regional co-ordinated hospital system. In visualizing such a system the picture has not been confined to hospitals provided for under the State Plan but has included all of the existing hospitals and future hospitals which may be constructed independently. As a part of this pattern on paper regional maps have been prepared showing the envisioned co-ordination among all of the facilities of each region; that is, lines of affiliation between base hospitals and intermediate or rural hospitals. The goal is to make small and moderate-sized hospitals more capable than they are at present of meeting the needs of their patients. This requires co-ordination among hospitals; it requires relationships among hospitals whereby the larger and more amply staffed and equipped hospitals will provide smaller hospitals with the knowledge, skills, and services which the latter, by themselves, are unable to provide.

It is common knowledge that in a large number of hospitals, particularly with smaller facilities, patient care is far below basic standards. The lack of an adequate State hospital licensure law and the attitude of complacency and apparent lack of interest in good professional staff organization on the part of doctors are held to be partly responsible for the existing shortcomings. The State Agency feels that its activities in hospital construction will not add to this problem; on the contrary, although it is impracticable for the State Agency to improve this situation by direct action in the case of hospitals not participating in the construction program, the impact of well-constructed, well-conducted hospitals operating under its supervision cannot fail to be felt by other hospitals which do not approach such standards.

Let us take a look at the existing situation in Texas which probably does not differ greatly from that in many other States. There are at present in Texas 227 general hospitals with a capacity of 30 beds or less owned privately by individuals,

partnerships or corporations. These hospitals furnished over 5,000 beds, or 27 per cent of the beds in the State. Their average capacity is 21 beds; 65 per cent of them have a capacity of 21 beds or less. From whom will these hospitals receive the incentive to adopt adequate standards and to affiliate themselves with a co-ordinated hospital system? It is well known that privately owned hospitals have been openly opposed to the passage of a State licensure law. It would be naive for one to believe that these hospitals will be easily influenced to participate until they are assured that their independence will not be infringed. They must be convinced that the ideal can be achieved without loss to the hospital of its individuality, initiative, or local responsibility.

There are at present in Texas 66 hospitals (not including those constructed under Public Law 725) owned by cities or counties, or by non-profit associations, with a bed capacity of 30 beds or less. How can these hospitals be stimulated to join in the movement for better patient care?

What existing agencies are doing anything about this now? The recent inauguration by the Texas State Hospital Association of regional conferences of hospital administrators for the purpose of discussing their mutual administrative problems and other subjects relating to better patient care is a step in the right direction. It is hoped that the value of these meetings will continue to be emphasized by the Association and that members of hospital boards and medical staffs will be encouraged to participate in them. Except for the activities of this Association and the program of the State Agency, there appears to be no organized group in the State which is making a serious region-wide attempt to improve patient care by raising the standards of hospital operation or by region-wide hospital affiliation. There are undoubtedly isolated instances of working consultative arrangements between hospitals but there appears to have been no general program developed. A number of city or county hospital councils have been organized by the larger city hospitals but their activities are largely limited to the problems of the local hospitals.

If the proposed pattern of integrated hospital services is to become a reality, the logical approach will be through the activities of regional hospital councils. A nucleus is already present in existing city hospital councils. These regional councils must be dedicated to the ideal of promoting the contribution of the hospital to better patient care by providing improved and more efficient clinical services, administration and educational opportunities through the co-operative efforts of urban and rural hospitals. To attain its inherent advantages, there must be understanding, vision, superior leadership and sustained interest in the objective.

Organization of the council should be dequate to carry out comprehensive plans. These should include:

1. Utilization of qualified professional personnel of urban areas to aid the hospital staffs of rural areas by arranging periodical visits to rural hospitals during which ward rounds, consultations and clinical conferences can be held, with discussions on timely clinical subjects of interest to busy doctors.
2. The provision of short-term postgraduate courses for doctors and nurses.
3. The provision of educational facilities in regional hospitals for technical personnel of outlying hospitals for refresher training, and for visits by qualified consultants for advice and checking of methods and results.
4. The provision of a similar type of consultation and guidance in administrative matters.

Ways and means for providing for the cost of employing the necessary personnel, travel expenses, etc., must be determined. Published estimates indicate an annual budgetary requirement for each council of from $50,000 to $75,000. It is conceivable that participating hospitals might be able to finance a project of reasonable magnitude. However, this is not a very promising solution as few hospitals will consider the financial outlay to be within their budgets.

A number of regional councils have been developed in other States with aid from philanthropic foundations. Among these might be mentioned the Council of Rochester Regional Hospitals which receives aid from the Commonwealth Fund; a program in New England aided by the Bingham Associates Fund;

another in Virginia aided by the Commonwealth Fund; the Duke Endowment program in North and South Carolina; and a Michigan program aided by the Kellog Foundation. These privately supported councils will, of course, furnish valuable lessons. Obviously this method of financing them can hardly be considered as a practical solution to the State-wide problem.

In October 1949, the Eighty-first Congress enacted Public Law No. 380 which amended the basic law authorizing activities of the Public Health Service to include:

"Studies and Demonstrations Relating to Co-ordinated Use of Hospital Facilities

Sec. 636. In carrying out the purposes of Section 301 with respect to hospital facilities, the Surgeon General is authorized to conduct research, experiments, and demonstrations relating to the effective development and utilization of hospital services, facilities, and resources, and, after consultation with the Federal Hospital Council, to make grants-in-aid to States, political subdivisions, universities, hospitals, and other public and private nonprofit institutions or organizations for projects for the conduct of research, experiments, or demonstrations relating to the development, utilization, and co-ordination of hospital services, facilities, and resources. Any award made under this section for such project in any fiscal year may include amounts not to exceed the four succeeding fiscal years, and such amounts for such succeeding fiscal years shall constitute contractural obligations of the Federal Government."

A large number of proposed projects and applications for grants under this amendment have been received by the Surgeon General. This type of research will furnish much needed light and inspiration for the practical application of programs of hospital service integration.

Each State must work out its own solution. It will require active support from medical schools, the State Medical Association, the State Hospital Association and from leaders in societies of specialists. The indorsement and counsel of the American Medical Association, the American College of Surgeons and the American Hospital Association should be sought.

It will require leadership of a high order and long-term planning.

A beginning has already been made in the peripheral approach by the State Agency in building well-constructed hospitals, organized and operated in accordance with accepted basic standards, and maintaining rational but limited consultative liaison with intermediate and base hospitals. As new intermediate and regional hospitals are constructed, or existing installations expanded, under the Hospital Construction Program, it is believed that much can be accomplished by indoctrinating these hospitals with the ideal of integrated hospital services so that their activities may be dove-tailed with those of the peripheral hospitals.

Summary

1. Relevant points in Public Law 725 are reviewed.

2. Problems of hospital planning and construction and a method of approach to the establishment of sound hospital organization for better patient care are discussed.

3. The chief obstacles to the standardization of small and medium-sized hospitals are (1) failure of the members of hospital boards to recognize and accept full responsibility, and (2) the complacency of doctors with regard to existing mediocre hospital facilities.

4. A program of integrated hospital services must be combined with a systematic effort to advance better hospital standardization if it is to be successful.

5. No satisfactory solution which is applicable to all States for the establishment of effective integration of hospital services can be avanced at present.

6. Progress is being made by State Agencies in stimulating hospitals within the State Hospital Construction Program to establish helpful relations between urban and rural hospitals.

ACKNOWLEDGMENT

Direct and indirect quotations have been freely utilized from informational documents published by U. S. Public Health Service.

THE EMERGENCY CARE, HANDLING, AND TRANSPORTATION OF THE INJURED AND ITS IMPORTANCE IN THE COMMUNITY

CARL B. YOUNG, JR.*

To begin this discussion on adequate emergency provisions for the injured, it is necessary to mention a few facts of which most of us are aware. The progress made in this country in conjunction with almost total mechanization has been accompanied by a proportionate increase in the number of severe and horrible accidents. The fast moving society in which we find ourselves also has its retaliations, primarily to the cardio-vascular system of man.

The Present Situation

As a general rule, the majority of accidental injuries occurs in the group under 40 years of age, while the larger percentage of victims afflicted with cardio-vascular diseases resulting in what may be termined an emergency situation, are in the group over 40 years of age. Among the leading causes of death in the United States, which are handled as emergency cases in most instances, are diseases of the heart, cerebral hemorrhage, and accidents. To combat this high mortality rate due to cardio-vascular disease there are educational programs in most communities which strive toward prevention by advising frequent medical examination and explaining the danger signs which mean trouble ahead. Of course, as man's life span increases, these deaths, due to the wearing out of the body cells, are in many cases a very natural thing.

In the field of accidents, the industrial safety divisions of the various plants and industries are carrying on an excellent program. The law enforcement agencies are doing a good job in the control of traffic accidents. The elimination of accidents on the farm and in the home is receiving the continuing support of the accident prevention programs of the National Safety

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*Prepared for the Sectional Meeting, American College of Surgeons, El Paso, Texas, February 13–14, 1950.

Council and the American Red Cross as well as those of other agencies.

We, as a nation, are aware of the toll of disease and accidents, and are striving to lessen this toll, if possible. Certain steps have been taken and we go forward with an ever-increasing drive to make our program more effective. Yet, regardless of how good our program of prevention is, and how well we work at it, the fact remains that we are still confronted with cases of sudden illness and injury from accidents and will continue to meet them as long as man exists on earth.

Realizing that we shall continue to have people subject to sudden illness, and many more subject to injury, *what provisions have been made to give these victims adequate emergency care on the scene of the accident, and until they reach the services of a physician or other medical attention?*

This paper is concerned with a survey on the First Aid care rendered to, and the practices of emergency transportation of seriously ill and injured patients in *those States representing this region.* The aim of this discussion is to gain further scrutiny on the important problem of how our injured are cared for. From a careful observation and extensive survey, there is ample evidence that the emergency care and transportation of the injured in this region is very inadequate. Therefore, the discussion will describe this inadequate care which the injured are now receiving, as well as that care which should be rendered.

To the casual observer, little can be detected which would lead one to believe that his fellow man was not getting the best emergency assistance. To the man having a background of medicine, health, athletics, scouting, or first aid training, an entirely different picture is apparent.

Emergency First Aid

Let us start with the arrival of the emergency ambulance. The driver gets out upon arrival at the scene of the accident and, together with his assistant, they remove the stretcher and move over to the victim. The first essential of emergency first aid is now required. This is an examination of the victim to ascertain as near as possible the extent of the illness or injury. Is there

hemorrhage which can be controlled? Is the victim breathing? Is the administration of oxygen indicated? Are there fractures or suspected fractures? These things cannot be determined unless an examination of the victim is carried out.

The examination of an injured victim is not difficult, and can be hurriedly yet thoroughly carried out. It is best done by the ambulance driver on one side and the attendant on the other, examining the victim from the head to the feet. Unfortunately, this primary essential of emergency first aid is seldom carried out by ambulance drivers and attendants. The victim would be fortunate indeed if he received even a hurried examination before being placed on the stretcher.

Next, after ascertaining the extent of the victim's injuries, the proper first aid must be rendered *on the spot*, before any movement of the victim is carried out. Naturally, in view of a situation which may result in additional injury to this victim or to his rescuers, removal to safety would come first. Such explosive and hazardous situations are not too frequently encountered.

Arrest of hemorrhage is the first procedure carried out in rendering emergency first aid. There are few cases of external hemorrhage which cannot be arrested by direct pressure, pressure points, or tourniquets as a last resort. Yet, the arrest of hemorrhage is seldom carried out at the accident scene by ambulance drivers and attendants.

First aid measures for the victim who is not breathing is the next essential of emergency first aid. The indicated first aid for such victims is the application of artificial respiration. This must be immediately carried out, on the spot, until the victim recovers or is pronounced dead by a physician. This simple first aid procedure, which is carried out by a little knowledge and one's two hands, saves hundreds of victims from an asphyxial death every year. However, there are many occasions when victims are rushed to the hospital by emergency ambulance, with no attempt made to carry out resuscitation measures. These victims never have a chance.

There are those victims which require oxygen inhalation while enroute to the hospital, yet it is a life-saving measure constantly neglected. Fire and Police Department rescue

squads conclusively prove the value of oxygen inhalation in a multitude of emergencies where there is respiratory distress. Oxygen inhalation while enroute to the hospital is of exceptional value to the cardiac patient.

The immobilization of fractures is the next major consideration. All broken bones which can be located should be immobilized. Any suspected fracture, especially of the neck and back, should be immobilized. For multiple fractures of the body, fractured neck, back, or pelvis, placing of the victim on a broken back board, or "fracture board" is the recommended method of emergency immobilization. Padded splints may best be used in the immobilization of extremity fractures. The fractured femur, however, is best immobilized by the application of fixed traction. To properly apply splints and induce immobilization is a first aid procedure which at times may prove difficult under emergency situations. Generally speaking, an ambulance driver and attendant can easily and quickly carry out immobilization. However, no immobilization of fractures is one of the most common emergency measures neglected, yet fractures are a major injury from the start and present the possibility of death, permanent disability, loss of earning power, and dependence on the community.

The common excuse always given "that we don't splint because it takes too long and we can be at the hospital in a minimum amount of time" is against all principles of emergency care. First of all, regardless of the proximity of the hospital, any fracture should be immobilized, and extreme care should be used to lift the victim on to the stretcher. A few extra minutes at this point will prove valuable later in all phases of medical and hospital care. Frequently, injury to the spinal column is not severe, but improper lifting and failure to immobilize the victim on a splint board may cause trauma, resulting in permanent paralysis. Another example is the instance of the fractured pelvis, where mishandling may result in injury to the bladder. By skillful immobilization of fractures on the accident scene, the victim will be in a much more favorable condition when he arrives at the hospital for medical treatment. As an example, the victim sustaining a fractured

femur, if properly cared for on the accident scene will, upon arrival at the hospital be in condition to receive more immediate care of his injury than the unfortunate individual admitted with severe hemorrhage and with the fractured femur doubled up under him, or hanging twisted over the side of the ambulance stretcher.

Transportation to the Hospital

The manner in which an injured person is lifted makes a great difference. The common practice of the ambulance driver and his assistant is to heave and strain, lifting the victim like a limp rag, without due regard for the victim's injuries. At this point many minor injuries are aggravated and become very severe and quite unnecessary. The wise ambulance driver, by using the help of bystanders, placing from three to five on each side and directing the lifting, can roll or slide the stretcher in under the victim. In this manner, the entire body has been lifted in unison and carefully placed on the stretcher. When the usually limited number of hospital beds, as well as nurses and physicians are concerned, this careful handling of a victim is a very important matter.

Speeding to the hospital with an injured victim is generally contra-indicated. There are few emergencies which call for speed to the hospital. Transportation which is accompanied by swerving, fast turning, sudden changes in speed and severe jolting only increases the pain, the injuries, and the degree of shock. Another important factor is the fright and anxiety which go with the wild race to the hospital, especially among those victims already badly injured. This is a major factor when transporting a victim suffering from cerebral hemorrhage, or one having an acute heart attack. A screaming siren and a wild ambulance ride increases the victim's anxiety. Both psychologically and physiologically this improper care is not conducive to good life-saving practice. A check over the daily newspaper almost invariably points out this fact.

Speeding has a second major danger, which is the possibility of crashing, injuring or killing those people who may fail to clear the street. A speed of twenty to thirty miles per hour is

adequate for almost any victim having an injury, be it major or minor. From the standpoint of public safety, it is certainly much safer.

The prime purpose for educating as many of the general public as possible in emergency first aid, is that they may bridge the gap between the time of the accident and the arrival of the ambulance or physician. Speed has its proper place, and it is here that speed is essential. The speed with which aid is given in cases of hemorrhage, arrest of respiration, and internal poisoning, is life saving when action is taken promptly by the trained man on the street.

It is a misconception of the general public that the faster the injured are transported to the hospital for medical care, the better chance there is for recovery. Unfortunately, many ambulance drivers do not know (or in some cases care) why they should drive at a reduced speed to protect the victim from further jolting or bumping.

Of equal importance is the elimination of those actions which a well-trained fellow citizen does not allow to happen to the nijured, such as the ministration from over-enthusiastic though obviously untrained person before an ambulance can be summoned. Yet, it is a common practice in many communities to place an injured victim into a Police Squad car and rush to the hospital.

Another major point in the present quality of emergency ambulance service, which is indeed deplorable, is the loading of the victim into the rear of an ambulance and not observing him while enroute to the hospital. Unfortunately this ill practice is commonly seen. About the only time a victim is accompanied in the rear of an ambulance is when such patient is maniacal or extremely hysterical.

From the case histories of those victims cared for by Fire Service Rescue Squads, the importance of constant observation of victims while enroute to the hospital is clearly brought out. It is a well known fact that even after successful resuscitation a victim may again cease to breathe before reaching the hospital. Again, a victim may be breathing when loaded, yet suddenly stop breathing somewhere along the road to the hospital.

Should such victims be unobserved by qualified attendants they may very easily expire. The unconscious victim demands constant observation.

Trauma, such as gun-shot injuries to the face, jaw, neck, or fractures of the skull, etc., make this unfortunate victim susceptible to unnecessary death by strangulation if placed unobserved on a stretcher and forgotten until arrival at the hospital.

As another example, the victim with a badly *fractured femur* may, while unobserved become nauseated and vomit. Often severe shock occurs and efforts to help himself may be impossible—death due to strangulation may be the result. This victim never had a chance to reach medical attention. In most localities where an assistant rides with the ambulance driver, he remains on the front seat after "loading" the victim, instead of riding in the rear with the victim.

There is another serious practice which is contrary, not only to good first aid procedure but inhumanitarian as well. This ill practice is the placing of a second patient on the floor of the ambulance instead of on a safe and suitable stretcher. Observation of ambulances unloading patients often bears out this statement. A careful investigation often shows that some emergency ambulances have stretchers which are welded or otherwise have fixed joints. It is impossible to place a patient on such a stretcher with comfort or safety.

Emergency Ambulances

Lack of first aid training for ambulance drivers in any community is a deplorable thing. To hire as an ambulance driver an untrained man without first giving him the advantage of basic first aid training is to disregard completely the welfare of the injured whom he will be handling. Worse still, is the employing of adolescents who desire the position more for the thrilling ride than for the meager salary and long hours required. This practice, however, is common. The training of the driver and attendant is the first important step necessary, if adequate care of the injured is to be expected. Every person delegated with the responsibility of caring for the injured should be trained in the Standard and Advanced first aid courses

of the American Red Cross. This training should be carried out under the leadership of an able and experienced instructor who understands thoroughly emergency first aid.

The size and construction of the emergency ambulances commonly used in this section of the country are themselves subject to sharp criticism. Generally speaking they are small "sedan delivery" type trucks which are too short for many an injured victim. The rear doors often jam the patient's legs as they are banged closed. It is virtually impossible to load a victim into these ambulances after a fixed traction splint has been applied, unless the stretcher is so turned and loaded that the patient's splinted extremity will extend over the front seat. The rear compartment where the patient rides is seldom adequately illuminated. Only in those larger vehicles, such as are commonly used as transfer ambulances, will adequate space, heating and illumination be found. These ambulances are so constructed with a partition between them and the drivers cab that cold drafts of aid do not rush over them on the way to the hospital. Also, this prevents to a great extent the anxiety and fright which often accompany the noise of a screaming siren.

Last, but of equal importance, is the equipment carried, such as splints for fractures of the extremities, a fracture board for fracture of the spine or hip, a suitable stretcher for carrying a second patient should this be necessary; dressings for wounds, and oxygen for victims suffering from respiratory depression. This equipment is simple in design, light in weight, takes up little space, and with planned and cumulative training, the amount of time required to gain the knowledge necessary to utilize this equipment is neglible. Such equipment is seldom seen on emergency ambulances. For adequate emergency assistance by ambulance attendants, some such equipment must be carried.

Community Responsibility

A careful study of the various surveys which have been made throughout the country relative to emergency ambulance service are extremely interesting. It is generally conceded that

every community has a moral obligation to give emergency care to the sick and injured within its jurisdiction. This does not mean a hit-and-miss system, but one which is well operated.

When a person is seriously ill or badly injured he wants only the best in medical care. As this victim is generally transported to medical attention by an emergency ambulance it might well be stated that his medical care begins with the arrival of such ambulance. This being the case, the question of how the injured are treated before reaching medical attention presents a serious problem to the medical profession.

It is a fact that the most expert team of physicians in the most elaborately equipped hospital are working at tremendous odds should they receive a patient who has been neglected at the accident scene and during transportation to the hospital. Such things as the arrest of hemorrhage, immobilization of fractures, and the administration of oxygen will bring the physician a patient which he can generally always treat more successfully.

That first aid, when properly carried out by well trained and qualified personnel, is a good thing is likewise a known fact. Rescue squads of the Fire Service prove this daily. Possibly the best explanation for the successful life-saving achievements of these well trained personnel is the fact that their only reward or gain is one of personal satisfaction or simply humanitarianism.

Unfortunately every community does not have the services of a Rescue Squad. Even in those which do, only special cases generally receive their benefit. The larger percentage of the injured from the street and highway are handled by the emergency ambulance. Should those who operate them be unethical, rough or careless, or not otherwise qualified to handle such cases, proper emergency first aid will not be rendered.

Why then, if first aid is a proven good, is it so neglected? It is not intended in this discussion to side-track the reasons involved. They are: (1) The fact that first aid is sold too cheaply; (2) A lack of community education and awareness to the need of first aid and adequate emergency ambulance service; (3) A

lack of interest in the community toward first aid and care of the injured; (4) And last, but by no means least, the influence of local politics.

What then is the most practical solution in obtaining an improved emergency ambulance service? This is a most important question, yet one exceedingly difficult to answer. The one point which should be made clear is "*THAT STATE LEGISLATION IS NOT THE ANSWER.*"

The answer is one which can be decided only by the local physicians and Medical Society. This is a medical problem, first and last. There will be no improvement until the physicians themselves desire it. The Medical profession, and it alone, can demand and obtain satisfaction from their community government.

How the emergency ambulance service is improved is a *local* responsibility. A good sound City Ordinance *sponsored* and *approved* by *local physicians* is often one suitable solution. However, this ordinance must have constant supervision and enforcement as well as a severe penalty clause.

There is no such thing in emergency ambulance service as a "gentleman's agreement." Only the passage of a sound City or local ordinance would assist materially in improving such service. This helps in ruling out the human element of placing the dollar bill first and human life second—as exists in many private businesses. *POLITICS HAS NO PLACE* in the *CARE OF AN INJURED OR SERIOUSLY ILL MAN.*

Municipal emergency ambulance service has no equal—if properly supervised by a member of the local Medical Society. Whenever possible, Municipal Service should be encouraged. The patient is given every consideration under this type of service. This is likewise important to the physician who will eventually care for him.

Regardless of what local action is ultimately taken, one important feature stands out. Emergency First Aid requires community team work, and this work is absolutely essential. Community team work depends on a good progressive educational program. This educational process may well start in the local Fire and Police Departments.

The Police Department must co-operate in every way, making the work of the ambulance driver much easier. It is essential that these police officers understand and appreciate the value of good first aid themselves. They should never rush the work at the accident scene, but allow all the time required in caring for and loading the victim. When the accident investigator arrives at the scene of the accident, as he so often does, long in advance of any ambulance, his immediate concern should be for the victim. With no severe hemorrhage and respiration normal he has only to keep the crowd away and the victim lying as is. In some departments the accident investigator actually does the majority of emergency work. The police officer is of great help to the ambulance driver when the victim is lifted to the stretcher. He can secure man-power from the crowd to line four or five on each side of the victim and be assured of careful handling as the stretcher is placed under the victim.

The next link in the team work is when the ambulance arrives at the hospital. The staff and personnel working in the receiving ward must likewise appreciate the value of lay first aid. If good splints have been applied, a little praise should be given as encouragement for future good work. If an error has been made, good constructive suggestions should be given. Good bandages and other equipment should not be needlessly destroyed. One of the greatest errors made is to tear off a well applied splint which in all probability could remain until after an X-ray. Unless explained to the astonished ambulance driver, his services will likewise cease to be as they should. How the victim is handled in transfer from ambulance stretcher to emergency room table, is an index to the training and supervision of the receiving room at any hospital. If the nurses and doctors go through these motions in a haphazard way, no better care of the injured can be expected from the ambulance driver who will handle the patient in like manner before arrival at the hospital. Good and proper example must be set at the hospital. To facilitate the work of the ambulance driver, a bandage and splint exchange should be established and maintained in the emergency ward.

The best system, regardless of how emergency ambulance service is handled, is that system which uses when necessary the emergency service division of the police department, if such exists, or the rescue and first aid company of the fire department. There are many occasions where specially trained personnel with special equipment are needed. In a community where only voluntary life saving crews are available, their services should be utilized and fully supported.

In conclusion, I sincerely hope that the thought-provoking statements presented in this talk may be beneficial and may contribute materially in improving the quality of ambulance service in those States representing this Section; such improvement is needed, and only you, the physicians of our communities, can solve this problem.

CALLING ATTENTION TO:

Items of Possible Interest to Our Friends

JUNE, 1950

Warm Weather Stock: F Bowers describes *Principles of Bibliographical Description* (Princeton Press, 49, 505pp, $10). JA Boyd brilliantly follows (*The Papers of Thomas Jefferson*, 1760–1776; Princeton Press, 50, 679pp, $10, 1st of 52 vols.) JF Dulles offers *War or Peace* (Macmillan, NY, 50, 274pp, $1). A Einstein anthologizes *Out of My Later Life* (Philosoph Libr. NY, 50, 282pp, $4.75). N Farson notes *Last Chance in Africa* (Harcourt, Brace, NY, 50, 381pp, $5). B Frechtman translates A Guide's *Notes on Chopin* (Philosoph Libr. NY, 49, 130pp, $3.8). DE Green & WE Knox edit excellent reviews on *Research in Medical Science* (Macmillan, NY, 50, 492pp, $6.5). S Harris well eulogizes J Marion Sims (1813–83) in *Woman's Surgeon* (Macmillan, NY, 50, 442pp, $5). HM Holden considers *The Human Nose in Physiology, Psychology, Art, Drama, Folklore* (*Noses*, World Publ, Cleveland, 256pp, $3.5). P Huard & M Montagne offer analysis of G Cuvier (1769–1832) & his times (*L'Ext-Orient Med* 1/179–259/49). J Hudnut stimulatingly discusses *Architecture & the Spirit of Man* (Harvard Press, Cambridge, 49, 301pp, $4.5). ME Keeton analyses *The Philosophy of Edmund Montgomery*, Scotch-Texian physician (1835–1911) husband of Elizabet Ney, sculpturess (Univ Press, Dallas, 50, 386pp, $5). J Labatut & WJ Lane discuss *Highways in Our National Life* (Princeton Press, 50, 506pp, $7.5). OW Larkin surveys *Art & Life in America* (Rinehart, NY, 49, 547pp, $8). Before his death, LD Lewis well biographed Captain Sam Grant (Little Brown, Boston, 50, 512pp, $6). G Liljestrand offers tribute to capillary physiologist August Krogh (1874–1949) (*Acta Physiol Scand* 20/109/50). K Macgowan studies *Early Man in the New World* (Macmillan, NY, 50, 260pp, $5). ET Mitchell exposits "meliorism", AP Brogan's theory of ethics, with historical account (*A System of Ethics*, Scribner, NY, 50, 559pp, $4). A Montagu discourses *On Being Human*, noting "ethicogenesis" (Schuman, NY, 50, 140pp, $2). P Popenoe says *Marriage Is What You Make It* (Macmillan, NY, 50, 221pp, $3). PA Schilpp edits detailed discussion on *Albert Einstein: Philosopher, Scientist* (Library Living Philosophers, Evanston, Ill, 50, 781pp, $8.5). CH Walker does well by *Eleanor of Aquitaine: Greatest of All French Women* (Univ N Carolina Press, Chapel Hill, 50, 420pp, $5). IB Wright has reservations on implications of optimism in expanding America (*Proc Amer Philosoph Soc* 94/18/50). *P P & BC:* B Andersson & Co suggest rhythmic excitation of chemoreceptors in carotids & aorta in hemorrhage as cause of Mayer waves in BP & Cheyne-Stokes breathing (*Acta Physiol Scand* 20/203/50). B Andersson & Co show superior laryngeal nerve stimulation in swallowing reflexly augments intestinal motility (*ibid*, p 253). HC Anderson & M McCarty describe use of C-reactive protein to estimate rheumatic activity (*Am J Med* 8/445/50). EW Boland reviews relation of adrenal cortex to

rheumatic disease (*Ann Rheum Dis* 9/1/50). W. C. Cutting edits 1st vol. *Ann. Rev. Med.* (Stanford, 50, 484pp, $6). N Dobrovloskaia-Zavadskaia says pyruvic acid accumulation in B_1 deficiency is responsible for symptons (pyruvism) *Rev Canad Biol* 9/28/50). F Duran-Reynals discusses neoplastic infection & cancer (*Am J Med* 8/490/50). N Feldberg & RCY Lin show formation of acetylcholine in gut is important in maintaining usual tone, motility & secretion (*J Physiol* 111/96/50). Food & Drug Administration please note that AE Feller & Co show antihistaminic drugs have no effect in preventing or curing common colds or undifferentiated repiratory diseases (*New Eng J Med* 242/737/May 11, 50). BA Houssay shows importance of sulphur cmpds in carbohydrate metabolism & diabetes (*Am J Med Sci* 219/353/50). EB Keller & Co show epinephrine methyl comes from methionine (*J Biol Chem* 183/627/50). J Kruszynski shows high Ca content of lymphocytes & monocytes (*J Physiol* 111/89/50). Z Maratka describes neurodigestive asthenia (*Gastroenterol* 75/228/50). G Milles & DB Dorsey suggest intra-uterine respiratory movements are factor in development of pulmonary vascular system (*Am J Path* 26/411/50). HD Moon & Co show pituitary growth hormone causes lymphoid peribronchial hyperplasia with high incidence of lymphosarcoma (*Cancer Res* 10/297/50). R Pitt-Rivers discusses mode of action of antithyroid agents (*Physiol Rev* 30/194/50). M Reiss & IDK Halkerston give pharmacologic characterization of ACTH (*J Pharm & Pharmacol* 11/236/50). AG Smith offers method of demonstrating nuclear chromatin of bacterial cells (*J Bact* 59/575/50). FF Yonkman edits full discussion of antihistaminic agents in allergy (*Ann NY Acad Sci* 50/1013–1208/50).

JULY, 1950

GENERAL: Good reading is JB Cleland's account of Australian medical naturalists (*Med J Austral* 1/549/Apr 29/50). A welcome biography is E Earnest's *S Weir Mitchell: Novelist & Physician* (Univ Pennsylvania Press, Phila, 50, 279pp, $3.5). English fair-play appears in publishing NI Nuzhdin's defence of Soviet genetics, which misses real point of free discussion, & gets J Huxley's reply (*Nature* 165/704, 731/May 6/50). W Pigman & EB Carmichael suggest ethical code for scientists (*Science* 111/643/June 16/50). A Rapoport & Co offer biobibliography of AHS Korzybski (1879–1950) (*Etc* 7/163/50). P Romanell asks *Can We Agree?* on scientific basis for ethics (Univ. Texas Press, Austin, 50, 110pp, $1). PB Sears discusses *Charles Darwin the Naturalist as a Cultural Force* (Scribner, NY, 50, 133pp, $2). TM Sonneborn wisely discusses heredity, environment & politics (*Science* 111/529/May 19/50). Note Symposium on Army Medical Library (*Bull Med Libr Asso* 38/75/50). A Vogl delightfully describes how the diuretic properties of organic mercurials were discovered by him in 1919 (*Am Hear J* 39/881/50). HB Vickery discusses origin of word "protein" by Berzelius in 1838 (Yale J Biol Med 22/387/50).

BASIC BIOLOGY: HW Ades & JM Brookhart analyse the central auditory pathway (*J Neurophysiol* 13/189/50; now have some fun with huge auditory paths of dolphins!) K Altmann experiments with mechanical factors in

bone building (*Ztschr Anat Entw* 111/457/50). F M Burnet & F Fenner revise *The Production of Antibodies* (Macmillan, London, 50, 210pp, $3). T Casperson offers important *Cell Growth & Cell Function: A Cytochemical Study* (Norton, NY, 50, 176pp, $5). FHC Crick & AFW Hughes note elastic properties of cytoplasm & gelling behavior (*Exp Cell Res* 1/37/50). E Geiger analyses time factor in protein synthesis (*Science* 111/594/June 2/50). Worthy is DHK Lee's *Physiology of Tissues & Organs* (CC Thomas, Springfield, Ill, 50, 172pp, $4). Ditto for Lane Lectures, W Penfield & T Rasmussen's *The Cerebral Cortex of Man* (Macmillan, NY, 50, 248pp, $6.5). SH Sturgis reviews mechanism & control of primate ovulation (*Fertil Steril* 1/40/50). Big & worthy is H Selye's *Stress* (Acta, Montreal, 50, 1000pp, $14).

CLINICAL: WB Ackermann & T Francis note biochemical aspects of herpes infection (*Proc Soc Exp Biol Med* 74/123/50). MW Bick & RM Wood discuss inhibiting effect of heparin on allergic reactions (*J Immunol* 64/357/50). TC Butler surveys theories of general anesthesia (*Pharmacol Rev* 2/121/50). JR DiPalma & JE Schults review antifibrillatory drugs (*Med* 29/123/50). M Dupont & A Ricard find histidine in urine of early pregnancy (*Presse Med* 58/535/50). EB Farnsworth finds help from ACTH in glomerulonephritis (*Proc Soc Exp Biol Med* 74/57/50). E Fforijn explains action of arsenite & urethane in leukemia & polycythemia on basis of interference with energy metabolism (*Acta Haematol* 3/268/50). R Frant & J Groen show mortality rate over 9 years of hypertension is thrice average (*Arch Int Med* 85/727/50). R Grandpierre & Co note paradoxic effects of oxygen administration in anoxia (*J Physiologie* 42/5/50). HH Kalbfleisch interprets AD Speransky's generalized pathology concept of the primacy of nervous system in morbidity (*Arzt Forsch* 4/232/May 10/50). K Lange & Co show 5 day injections of heparin needed to prevent frost-bite gangrene (*Proc Soc Exp Biol Med* 74/1/50). CD Marple & IS Wright discuss *Thromboembolic Conditions & Their Treatment with Anticoagulants* (CC Thomas, Springfield, Ill, 50, 416pp, $8.5). H Molitor & OE Graessle review pharmacology & toxicology of antibiotics (*Pharmacol Rev* 2/1/50). PM Neuda describes expulsion crescent, a new pathological red cell figure associated with cirrhosis of liver (*Proc Soc Exp Biol Med* 74/27/50). M Perrin describes acute & chronic heart pathology in Chagas disease (*Arch Inst Cardiol Mex* 20/31/50). RF Pitts & OW Sartorius discuss action & use of diuretics (*Pharmacol Rev* 2/161/50). PF Salisbury & JH Miller show cross transfusion in acute mercury nephrosis must be underway within an hour to be effective (*Proc Soc Exp Biol Med* 74/17/50). F Sargent notes value of electromyography in clinical neurology (*Lancet* 1/937/May 20/50). P Scholmerich & G Hildebrandt study water content of skin in relation to skin temperature (*Ztschr ges Exp Med* 115/558,570/50). SV Sivilla & JJ Vargas confirm anoxic pumonary vasoconstriction & indicate reflex factors involved (*Rev Exp Fisiol* 5/271/50). F Venulet & R Kadlubowski show antihistamine action of bile (*Pol Tyg Lek* 5/201/50). F Verzar notes metabolic effects of adrenocortex hormones (*Schw Med Wchnschr* 80/468/May 6/50).

AUGUST, 1950

CLINICAL: Better get *Atomic Medicine* edited by CF Behrens (Nelson, NY, 5, 461pp, $7.5). A Dowzenko & M Krokowska find transcient lymphocytosis after convulsive electroshock (*Pol Tyg Lek* 5/281/May 20, 50). KAJ Jarvinen analyses contrary relations of rheumatoid arthritis & rheumatic fever to allergy (*Ann Med Int Fenniae* Suppl 5/50/99pp). L Kirschner & DJA Gallagher show antistreptolysin titre is indicator of streptococcal infection & rheumatic fever (*N Zealand Med J* 49/118/59). K Linck finds alcohol lethal to humans (125 cases) in doses of 2 to 3.5 gm/kg, with blood levels 0.4 to 0.6%, & death about 8 hrs after last intake (*Med Klin* 45/570/May 5/50). JR Moreton shows atherscleresis comes from large particle size of newly absorbed lipids & proposes fat tolerance test (*J Lab Clin Med* 35/373/50). SR Rosenthal shows safety & value of BCG vaccination (*J Pedi* 36/399/50). K Schneider & E Kruse recommend pectin-$BaSO_4$ for gastro-enteric radiography (*Artz Forsch* 4/280/May 25/50). R Tagnon & J Corvilain confirm JH Laragh & TP Almy (*Proc Soc Exp Biol Med* 69/499/48) that insulin hypoglycemia reduces eosinophils except in adrenal & pituitary insufficiency (*Acta Clin Belg* 5/134/50). P Vallery-Radot & Co find allergic asthmatic attack due to sudden bronchial edema from antigen-antibody reaction (*Presse Med* 58/661/June 14/50). W Volland & Co suggest hypercupraemia as factor in melanosis (*Artz Forsch* 4/242/May 10/50). H Zilliacus discusses venous thrombosis & intravascular aggregation of red cells in inflammation & cold (*Acta Chirur Scand* 99/407/50).

OBSTETRICAL: H Alvarez & R Caldeyro find rhythmic human uterine contractions in pregnancy with increasing intensity but slower frequency as labor develops (*Surg Gyn Obs* 91/1/50). P Bergman shows temperature changes correlate best with sexual cycle & ovulation (*Acta Obs Gyn Scand* 29/Suppl 4/139pp/50). S Parviainen & Co find desoxycorticosterone poisoning similar to toxemia of pregnancy (*Ann Chir Gyn Fenniae* 39/Suppl 1/33pp/50).

BASICAL: JT Aitken notes nerves implanted in denervated muscles form functioning motor end-plates (*J Anat* 84/38/50). LT Fairhall digests *Industrial Toxicology* (Wms & Wilkins, Baltimore, 50, 494pp, $6). BN Halpern & Co show antihistaminics prevent pulmonary edema from inhalation of phosgene (*Arch Internat Pharmaco Therap* 82/425/50). H Hoseman offers *Die Grundlagen der Statistischen Methoden fur Medizinen* (Thieme, Stuttgart, 50, 131pp, DM 14.8). MA MacConail continues analysis of movements of bones & jts, considering function of synovial fluid & inarticular discs (*J Bone Jt Surg* 32/244/50). EP Ralli edits *Adrenal Cortex: 1st Conference* (Macy Edition, NY, 198pp, $2). H Selye reviews stress & adaptation O_2 consumption increased by calcium lack & potassium excess, while consumption increased by calcium lack & potassium excess, while calcium calcium & magnesium excess depress (*Rev Belge Path Med Exp* 2/22/50). AV Wolf discusses *Urinary Function of the Kidney* (Grune & Stratton, NY, 50, 372pp, $7.50).

GENERAL TURMOIL: RS Aitken writes well on medical standards professionally (*N Zealand Med J* 49/97/50). Niels Bohr bravely asks UN to promote free interchange of scientific information between nations (*Science* 112/1/July 7/50). GB Darling asks "Can We Pay for Our Medical Schools"/ (*Atlantic*, June, 50). G Koch biobibliographies A Egas Moniz, Nobelate leucotomist (*Artz Forsch* 4/261/May 25/50). I Langmuir discusses *Phenomena, Atoms & Molecules* (Philosoph Libr, NY, 50, 436pp, $10). RA Millikan autobiographizes (Prentice-Hall, NY, 50, 311pp, $4.5). ED Mills describes medical survey of Taiwan (*Am Bur Med Aid China*, NY, 11pp, May 24/50, free). HJ Morganthau well analizes the case for negotiating with Russia, noting that observance of agreements depends on national interests (*Bull At Sci*, 6/143/50). CB Palmer says it's *Slightly Cooler in the Country* (Doubleday, NY, 50, 222pp, $2.5). RA O'Brien asks if we are using medical history wisely (*Med J Austral* 1/649/May 20/50). E Rabinowitch edits *Minutes to Midnight: The International Control of Atomic Energy* (Bulletin Atomic Scientists, Chicago, 50, 128pp, $1). L Yutang puts the case for sentiment (*Sat Rev Lit* July 8/50, p7).